Program Nr: 1 from 2003 ASHG Annual Meeting

Targeting of distinct human genomic sequences by male and female meiotic recombination. *S. Dolan[1], Y. Deng[1], M. Codyre[2], M. Cruz[3], P.E. Cohen[1], M. Fazzari[1], J.M. Greally[1].* 1) Albert Einstein College of Med, Bronx, NY; 2) Aliquo Bioinformatics, Queens, NY; 3) The American Association for the Advancement of Science, Washington, DC.

Meiotic recombination rates vary within chromosomes and between the sexes. The non-random distribution of recombination sites appears to be influenced by a variety of factors, including DNA sequence composition, in mammalian and yeast genomes. We used linear regression to identify the target sequences for recombination in the human genome. We found that both germ lines avoid regions enriched in L1 long interspersed nuclear elements (LINEs), male recombination targets the genomic compartment defined by high gene and CpG island frequencies and Alu short interspersed nuclear element (SINE) accumulation, whereas the female germline targets distinct regions enriched in a group of co-varying transposable elements, MIR SINEs, MaLR LTRs and both MER1 type and AcHobo DNA elements. We determined that recombination in the male germline selects preferentially a telomeric subset of potential target sequences, whereas female recombination utilizes all potential target sequences equally. As L1 LINEs are methylated in both germlines, Alu SINEs are more methylated in the female than the male germline and subtelomeric regions are hypomethylated in human sperm, the patterns of sequence features observed suggest an inverse correlation between the degree of methylation and recombination frequency. Epigenetic influences, already recognised to be important in fungi, may therefore direct the targeting of recombination during human meiosis.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 2 from 2003 ASHG Annual Meeting

Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway. *P. Jin[1], S. Ceman[1], D.C. Zarnescu[2], M. Nakamoto[1], J. Mowrey[1], T.A. Jongens[3], D.L. Nelson[4], K. Moses[2], S.T. Warren[1].* 1) Dept Human Genetics, Emory Univ Sch Medicine, Atlanta, GA; 2) Dept Cell Biology, Emory Univ Sch Medicine, Atlanta, GA; 3) Dept Genetics, Univ of Pennsylvania Sch Medicine, Philadelphia, PA; 4) Dept Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Fragile X syndrome is caused by the loss of the fragile X mental retardation protein (FMRP). FMRP is a selective RNA-binding protein which forms a messenger ribonucleoprotein (mRNP) complex that associates with polyribosomes. Recently, mRNA ligands associated with FMRP have been identified. However, the mechanism by which FMRP regulates the translation of its mRNA ligands remains unclear. MicroRNAs are small noncoding RNAs involved in translational control. Here we show that the human fragile X mental retardation protein (FMRP) complex co-immunoprecipitates with components of the RNA-induced silencing complex (RISC), including the mammalian ortholog of Argonaute protein, eIF2C2, and Dicer, as well as microRNAs. To examine the physiological importance of this association, using *Drosophila*, we rescue the phenotype caused by the overexpression of the FMRP ortholog by reducing the expression of eIF2C2 ortholog, *AGO1*. Our results suggest that FMRP may regulate the translation of its mRNA targets through the associated miRNAs and provide the first link between miRNA pathway and human disease.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 3 from 2003 ASHG Annual Meeting

Mutations in Inversin cause Nephronophthisis type 2, linking cystic kidney disease to the function of primary cilia and left-right axis determination. *E. Otto*[1], *B. Schermer*[2], *T. Obara*[3], *J.F. O'Toole*[1], *D. Landau*[4], *J.A. Goodship*[5], *T. Strachan*[5], *C. Antignac*[6], *G. Walz*[2], *I.A. Drummond*[3], *T. Benzing*[2], *F. Hildebrandt*[1, 7]. 1) Dept Ped, Univ Michigan, Ann Arbor, MI; 2) Renal Division and Center for Clinical Research, Univ Hospital Freiburg, Germany; 3) Renal Unit, Massachusetts General Hospital and Dept of Medicine, Harvard Medical School, Charlestown, MA; 4) Dept Ped, Soroka Medical Center, Beer Sheva, Israel; 5) Univ of Newcastle, Inst Human Genetics, International Center for Life, Newcastle upon Tyne, United Kingdom; 6) Dept Genetics and INSERM U574, Necker Hospital, Ren Descartes Univ, Paris, France; 7) Dept Human Genetics, Univ Michigan, Ann Arbor, MI.

Nephronophthisis (NPHP), an autosomal recessive cystic kidney disease, constitutes the most frequent genetic cause for end-stage renal failure in children and young adults. The genes causing NPHP types 1 and 4 have been identified, and a gene locus for infantile nephronophthisis (NPHP2) was mapped to chromosome 9q21-q22. The kidney phenotype of NPHP2 combines clinical features of NPHP, and of polycystic kidney disease (PKD). We identified inversin (*INVS*) as the gene causing NPHP2 with and without *situs inversus*. We demonstrated the interaction of inversin with the *NPHP1* gene product nephrocystin suggesting that these two proteins participate in a common pathway. Furthermore, we identified interaction of nephrocystin with -tubulin, a major component of primary cilia. We are able to show co-localization of nephrocystin, inversin, and -tubulin in primary cilia of renal tubular cells. Additionally, we found NPHP1 and NPHP4 expression in cilia on the ventral side of the mouse node which are known to be implicated in left-right axis determination during embryonic development. Furthermore, we produced a PKD-like renal cystic phenotype and randomization of heart looping by knockdown of inversin expression in zebrafish, which was rescued with a wildtype murine inversin mRNA. The interaction and co-localization in cilia of inversin, nephrocystin, NPHP4 and -tubulin connects several pathogenetic aspects of NPHP to PKD, primary cilia function and left-right axis determination.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

IRF6 is a major modifier for nonsyndromic cleft with or without cleft palate. *T. Zucchero[1], M. Cooper[2], D. Caprau[1], L. Ribiero[3], Y. Suzuki[4], K. Yoshiura[5], K. Christensen[6], L. Moreno[1], M. Johnson[1], L. Field[7], Y. Liu[8], A. Ray[9], B. Maher[2], T. Goldstein[2], A. Lidral[1], S. Kondo[1], B. Schutte[1], M. Marazita[2], J.C. Murray[1].* 1) Univ Iowa; 2) Univ Pittsburgh; 3) Univ Sao Paulo, Brazil; 4) Aichi-Gakuin Univ, Japan; 5) Nagasaki Univ, Japan; 6) Univ Southern Denmark; 7) Univ British Columbia, Canada; 8) Zhabei Institute, China; 9) Univ Toronto, Canada.

We reported that mutations in Interferon Regulatory Factor 6 (IRF6) cause the most prevalent form of syndromic cleft lip and palate, Van der Woude syndrome (VWS). VWS has a phenotype similar to non-syndromic cleft lip with or without cleft palate (NSCLP) and NS cleft palate alone (NSCP), with the only additional feature being pits in the lower lip. To determine if IRF6 plays a role in NSCLP and/or NSCP, we evaluated a SNP identified within the coding sequence of IRF6 that changes a highly conserved amino acid, V274I. This SNP was genotyped on members of 3,530 nuclear families from 1,719 extended kindreds with ancestry in Europe, India and East Asia. Transmission disequilibrium (TDT) analysis revealed highly significant overtransmission of the V allele of this SNP from parents to NSCLP affected children ($p<10^{-9}$), but not NSCP ($p=0.99$). This association was strongest in Asian and weak or absent in some Caucasian groups. 287 Filipino NSCLP triads were genotyped for V274I plus 10 additional SNPs in the region. Haplotypes were generated using the EM algorithm, and TDT analysis revealed a strong positive association with the most common haplotype ($p<10^{-5}$). The associated haplotype extends out 200kb 3' of IRF6. There was also significant linkage disequilibrium in the region (D' ranging from 0.23 to 1.0). Sequencing of the entire 25 kb IRF6 genomic region in 24 individuals affected with NSCLP has thus far revealed 58 variants, 16 of which are etiologic candidates. Our results provide a resource for direct mutation identification and for the first time identify a major modifier for NSCLP. Studies of IRF6 have significant implications for genetic counseling, especially in assessing the risk of recurrence of NSCLP in a family.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 5 from 2003 ASHG Annual Meeting

Overexpression of MeCP2 causes a progressive neurological disorder in mice. *A.L. Collins, J.M. Levenson, B. Antalffy, A. Vilaythong, J.D. Sweatt, D.L. Armstrong, J. Noebels, H.Y. Zoghbi.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Rett syndrome, a leading cause of developmental disability in females, is marked by an apparently normal 6-18 months of development followed by regression, leading to mental retardation, autism, seizures, and stereotypical hand wringing. It is caused by mutations in the X-linked gene encoding Methyl-CpG-Binding Protein 2 (MeCP2). It is clear that Rett syndrome is caused by loss of the normal function of MeCP2, given that several mutations truncate the protein or impair its methyl-CpG-binding activity. However, the consequences of a gain of function of MeCP2 are not known. Therefore, in order to develop a tool that will allow us to further study the function of MeCP2 and to know if replacement therapy can be considered as a therapeutic option, we set out to evaluate the *in vivo* effects of MeCP2 overexpression. A P1-derived artificial chromosome (PAC) clone containing the entire human genomic locus of *MECP2* was identified and characterized. This PAC (671D9) contains all of the known regulatory regions of MeCP2 and no other known genes. Two independent transgenic lines were generated. The levels of MeCP2 were approximately 5X the endogenous protein in the more highly expressing line with an expression pattern paralleling that of the endogenous gene. Mice from both lines appear healthy for the first few months of life, but then develop a progressive neurological syndrome. Mice from the higher expressing line begin showing abnormalities around 10-12 weeks when they manifest behavioral abnormalities and develop abnormal forepaw movements. As the mice age, they develop seizures as well as abnormal spike and wave patterns on EEG analysis associated with nonconvulsive seizures. The mice gradually deteriorate, become severely hypoactive and ~40% die by 40 weeks of age. These data demonstrate that levels of MeCP2 must be carefully regulated, as modest overexpression is detrimental. Future studies will focus on the mechanisms by which MeCP2 overexpression induces the neurological phenotype and its relationship to the pathogenesis of Rett Syndrome.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 6 from 2003 ASHG Annual Meeting

Rett patients with both MECP2 mutations and 15q11-q13 rearrangments. *A. Renieri[1], C. Pescucci[1], I. Longo[1], F. Ariani[1], I. Meloni[1], M. Zappella[2], L. Russo[3], T. Giordano[3], G. Neri[3], F. Gurrieri[3].* 1) Department of Molecular Biology, Medical Genetics, University of Siena, Siena, Italy; 2) Child Neuropsychiatry, University hospital of Siena, Siena, Italy; 3) Medical Genetics, Universitá Cattolica del Sacro Cuore, Roma.

Rett syndrome (RTT) and autism (A) are classified as separate disorders. However, recently the identification of the Preserved Speech Variant (PSV) of RTT, where autistic behavior is usual, stressed the phenotypic overlapping between these two conditions. In 1999, it has been found that about 80% of RTT cases are due to a de novo mutation in the transcriptional silencer *MECP2* gene, classifying RTT as a monogenic X-linked dominant disorder. On the other hand, we and others have provided evidence that 15q11-q13 maternally inherited duplications are found in about 1% of autistic patients, suggesting that an abnormal dosage of gene(s) within this region might be responsible for A.

Based on clinical overlap between RTT and A, we decided to investigate RTT patients for the presence of 15q11-q13 rearrangements by testing a panel of SNPs covering this region. Now we show that a proportion of RTT patients (about 5%) present both a *MECP2* mutation and a 15q11-q13 rearrangement. Therefore, we hypothesize a digenic model in which a *MECP2* mutation is necessary but not sufficient to cause RTT phenotype in females based on the status of a second gene which, at least in a proportion of cases, may be the abnormal dosage of one or more 15q11-q13 genes. According to this model, RTT phenotype would result from the contemporary presence of a *MECP2* mutation and a less functional variant of a second gene. In support of our model, there is the identification of cases in which *MECP2* mutations remain silent in carrier females but cause X-linked mental retardation in their sons. The accurate characterization of 15q11-q13 region in order to identify genes potentially involved in neural development is presently ongoing.

In summary, we provided preliminary evidence that A and RTT (clinically related by long time) may have a common overlapped molecular basis.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 7 from 2003 ASHG Annual Meeting

A fragile X mental retardation protein interacting protein maps to the proximal breakpoint of the Prader-Willi syndrome common deletion region. *Y. Jiang[1], K. Wakui[1], Q. Liu[1], C.D. Kashork[1], J. Lehoczky[2], L.G. Shaffer[1], A.L. Beaudet[1]*. 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept Human Gen, Univ. of Michigan.

Prader-Willi syndrome(PWS) is a neurological disorders characterized by neonatal hypotonia, childhood obesity, hypogonadism, mental retardation, and behavioral problems. It is widely accepted that PWS is a contiguous gene deletion syndrome and deficiency of 15q11-q13 with paternal origin is the genetic defect for PWS. However, the molecular mechanism underlies the PWS remains elusive. Interestingly, there are multiple reports describing the PWS like phenotypes in fragile X syndrome patients who have full mutations in *FMR1* gene. We have done extensive genomic sequence annotation for the PWS candidate region.A sequence based BAC contig with extensive coverage of STS markers and transcripts were constructed and represents an updated version of physical map covering the PWS candidate region. We have refined the physical map of proximal breakpoint and mapped the deletion breakpoint precisely to a BAC clone in which LCRs were also revealed. We have characterized two protein coding genes, *PWSR1* and *PWSR2*, from the proximal breakpoint but within the common PWS/AS deletion interval. Biallelic expression for *PWSR1*and *PWSR2* was revealed from the imprinting analysis using RNAs from lymphoblasts and brain tissues of PWS patients. No allelic specific methylation was found to be associated with the CpG island of the *PWSR1*gene but PFGE analysis revealed a polymorphic genomic region adjacent to the breakpoint. Interestingly, *PWSR1* is identical to a recent reported fragile X mental retardation interacting protein CYFIP1. The function of *PWSR1* in growth of dendritic spines was suggested because of its interaction with Rac1. Moreover, a defect of dendritic spine growth was the consistent defect found in *Fmr1* null mice. This finding provides a plausible molecular link for a well documented clinical observation that a subset of fragile X patients exhibited the PWS like phenotype. We have obtained germline transmission of a null mutation for *Pwsr1* in mice. Null mutation of *Pwsr1* in mice appeared to be embryonic lethal.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 8 from 2003 ASHG Annual Meeting

Prevalence of premutation and intermediate alleles among patients referred for fragile X carrier testing. *A. Cronister, J. Teicher, T. Custer, E.M. Rohlfs.* Genzyme Genetics, Westborough, MA.

Fragile X syndrome (FXS) is a leading cause of inherited mental retardation (MR), with a carrier frequency of ~1 in 260 women. Screening MR individuals is effective in identifying affecteds yet the majority of carriers remain undiagnosed and unaware of reproductive risks. The aim of this study was to report our laboratory's experience with FXS carrier testing and to evaluate different carrier testing strategies by comparing carrier and intermediate allele detection rates. This study included 5757 women referred for FXS carrier testing from Jan 2002 to May 2003. Testing was performed by Southern blot and PCR analysis. Women were grouped as follows: 1) females with a family history (FH) of FXS (n=122) 2) females with a FH of unexplained MR, autism, learning disability or behavioral problems (n=1172) 3) females with premature ovarian failure (POF) (n=17) and 4) females with no significant FH suggestive of FXS (n=4446). Among females with a FH of FXS, 30 had a premutation (55-200 repeats) and 1 had a full mutation (>200 repeats) for a carrier frequency of 1 in 4. Among women with a suspicious FH, the premutation carrier frequency was 1 in 107, significantly higher than the carrier frequency in women with no FH (1/370 p=0.00364). Among women with POF, the carrier frequency was 1 in 6. The frequency of intermediate alleles (45-54 repeats) was not significantly different among women with a FH of FXS (1/31) vs. either those with a suspicious FH (1/62), or no FH (1/59) (p=0.080 and p=0.129). Mother-offspring transmissions were examined among 40 women with intermediate alleles. 17 transmitted the normal allele, 20 transmissions remained stable, 1 contracted (47 to 46) and 2 expanded minimally from 51 to 52 and from 52 to 55. Conclusions: 1) All testing strategies, including screening women with no significant FH, are successful in identifying premutation carriers. 2) In this data set, none of the intermediate alleles expanded to a full mutation; however, one expanded to a premutation. 3) Given similar intermediate allele frequencies among all groups, identification of intermediate alleles should not be seen as a deterrent to general population carrier screening.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 9 from 2003 ASHG Annual Meeting

Association of FMR1 CGG repeat size, X-inactivation ratio and transcript level with cognitive performance among women. *E. Allen, R. Letz, S. Sherman.* Depts. of Human Genetics and Epidemiology, Emory Univ, Atlanta, GA.

The fragile X syndrome (FXS) is the most common form of inherited form of mental retardation and is caused by the hyperexpansion and hypermethylation of a CGG repeat tract in the 5 untranslated region of the FMR1 gene. This methylation of the full mutation allele causes the gene to be transcriptionally silenced. In addition to the common allele form with <41 repeats, there are two other allelic forms of the FMR1 gene that are unmethylated and sometimes unstable when passed from parent to child: premutation (61-200 CGG repeats) and intermediate (41-60 CGG repeats). Recently, specific phenotypes not related to the FXS have been reported to be associated with premutation alleles only, not full mutation alleles: a 20 fold increased risk for premature ovarian failure (POF) among female carriers and an increased risk for a tremor ataxia syndrome (TAS) among older male carriers. Studies of neuropsychological measures have been equivocal. We have ascertained non-clinically referred women, ages 18-50 years, with common (n=70), intermediate (n=67) and premutation (n=32) alleles and administered a comprehensive neuropsychological test battery. As an initial analysis, we have examined the Full, Verbal and Performance scales of the WAIS-III using linear regression with FMR1 repeat size defined as the exposure variable. After adjusting for racial group (Caucasian and Other) and age, we found a significant linear relationship of Verbal IQ (p=.001). The effect size between the common and premutation carrier groups using the estimated marginal means with observed standard deviations from the common allele group was -0.58. We are currently investigating the relationship of FMR1 transcript level and X-chromosome inactivation ratio with this outcome measure. In previous studies increased levels of FMR1 transcript have been observed among premutation carriers compared with non-carriers. Variation in transcript level may better predict the decrease in Verbal IQ compared with only CGG repeat size. Our initial studies show that transcript levels among women increase linearly with repeat size; repeat size explains about 7% of the total variance.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Penetrance of the fragile X-associated tremor/ataxia syndrome (FXTAS) in a premutation carrier population: Initial results from a California family-based study. *S. Jacquemont*[1], *R.J. Hagerman*[1], *M.A. Leehey*[2], *D.A. Hall*[2], *R.A. Levine*[3], *J.A. Brunberg,*[4], *L. Zhang*[5], *T. Jardini*[1], *S.W. Harris*[1], *K. Herman*[1], *E. Berry-Kravis*[6], *F. Tassone*[7], *P.J. Hagerman*[7]. 1) MIND Inst, Univ California, Davis Med Ctr, Sacramento, CA; 2) Dept Neurology, Univ of Colorado Health Sciences Center; 3) Dept of Mathematics and Statistics, San Diego State Universty; 4) Dept Radiology, Univ California, Davis, Med Ctr; 5) Dept Neurology, Univ California, Davis, Med Ctr; 6) Dept Pediatrics, RUSH-Presbyterian-St. Lukes Medical Center, Chicago, IL; 7) Dept Biological Chemistry, Univ California, Davis, School of Medicine, Davis.

Several articles have recently delineated a new neurodegenerative disorder named FXTAS (fragile X Tremor Ataxia Syndrome) which affects older male carriers of the FMR1 premutation allele. The main clinical features are cerebellar ataxia and/or intention tremor. Additional symptoms include, cognitive decline, parkinsonism, peripheral neuropathy, and autonomic dysfunction. This family-based study evaluated 186 individuals. The data were collected through a survey and a standardized neurological exam including the 3 main movement disorder rating scales which were videotaped and subsequently scored in a blinded fashion. Data from the survey demonstrated an age-related penetrance of probable and definite FXTAS in male carriers: 15%, 30%, 50% and 75% (lower-bound estimates) for subjects in their fifties, sixties, seventies, and eighties, respectively. The male carrier group had a 24-fold increased risk ($p=0.003$) of combined intention tremor and gait ataxia when compared to age-matched male controls. The clinical data demonstrated that male carriers experienced more difficulties on all neurological rating scales when compared to controls ($p<0.05$). The study demonstrates that the cumulative penetrance of FXTAS is high. Since male premutation carriers are frequent (~1/813 males) in the general population, older males with ataxia and intention tremor should be screened for the FMR1 mutation.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Survey of brain-expressed genes in a 7.3 Mb region on proximal Xp involved in non-syndromic X-linked mental retardation. *L.R. Jensen*[1], *V. Kalscheuer*[1], *K. Freude*[1], *U. Gurok*[1], *S. Haesler*[1], *O. Hagens*[1], *B. Aranda*[1], *N. Hartmann*[1], *T.C. Roloff*[1], *S. Shoichet*[1], *J. Tao*[1], *A. Tzschach*[1], *B. Moser*[1], *J. Chelly*[2], *C. Moraine*[3], *J.P. Fryns*[4], *H. Yntema*[5], *M. Partington*[6], *H.H.R. Ropers*[1], *S. Lenzner*[1]. 1) MPI for Molecular Genetics Berlin, D; 2) INSERM U129-ICGM, Faculte de Med. Cochin, Paris, F; 3) INSERM U316, Service de Genetique, Tours Cedex, F; 4) Center for Hum. Gen., Univ. Hospital Leuven, B; 5) Dept. of Hum. Gen., Univ. Hospital Nijmegen, NL; 6) Univ. of Newcastle, NSW, AU.

Severe mental retardation (MR) affects about 0.5% of the population in Western countries. Genetic disorders account for roughly two thirds of these cases, and 25% of these are thought to be due to mutations in X-linked genes. More than 130 different syndromic forms of X-linked mental retardation (S-XLMR) have been defined, and the molecular defect has been found in >30 of these. Non-syndromic XLMR (NS-XLMR) is more frequent than S-XLMR, but due to its extensive heterogeneity, elucidation of the underlying genetic defects has lagged behind. Analysis of linkage intervals in 125 families with NS-XLMR has shown that >30% of these gene defects are clustered on proximal Xp and in the pericentric region (Ropers et al., TIGS 19: 316, 2003). In this study we have employed DHPLC to screen 30 families with overlapping linkage intervals for mutations in 49 brain-expressed genes, all located within a 7.3 Mb segment of Xp11 flanked by ELK1 and ALAS2. So far, a total of 62 different sequence variants were found in these genes, 39 located in non-coding and 23 in coding regions, respectively. Having excluded those sequence variants also present in healthy male controls (polymorphisms) the sequence variants in the coding regions comprise several frame-shift mutations in the PQBP1 gene (see Kalscheuer et al., this issue), a splice site mutation resulting in skipping of one exon (see Freude et al., this issue) and eight different missense mutations in six other genes. Together, these mutations account for 13 of the 30 families examined. Screening of 20 additional brain-expressed genes from this region is in progress and may shed light on the molecular causes of up to one third of all cases with NS-XLMR.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Mutations in the polyglutamine-binding protein 1 gene cause X-linked mental retardation. *V.M. Kalscheuer*[1], *K. Freude*[1], *L.J. Jensen*[1], *L. Musante*[1], *H.G. Yntema*[2], *J. Gécz*[3], *A. Sefiani*[4], *H. vanBokhoven*[2], *G. Turner*[5], *J. Chelly*[6], *C. Moraine*[7], *J.P. Fryns*[8], *U. Nuber*[1], *M. Hoeltzenbein*[1], *C. Scharff*[1], *H. Scherthan*[1], *S. Lenzner*[1], *B. Hamel*[1], *S. Schweiger*[1], *H.H. Ropers*[1]. 1) Max-Planck Institute for Molecular Genetics, Berlin, Germany; 2) Department of Human Genetics, University Medical Centre, Nijmegen, The Netherlands; 3) Women's and Children's Hospital and The University of Adelaide, Adelaide, Australia; 4) Departement de Genetique et de Biologie Moleculaire INH, Rabat, Morocco; 5) Hunter Genetics and University of Newcastle, P.O. Box 84, Waratah, New South Wales, Australia 2298; 6) Institut Cochin de Genetique Moleculaire, CNRS/INSERM, CHU Cochin 75014 Paris, France; 7) Services de Genetique -INSERM U316, CHU Bretonneau, Tours, France; 8) Center for Human Genetics, Clinical Genetics Unit, Leuven, Belgium.

X-chromosomal gene defects are an important cause of mental retardation. As recently shown, many of the underlying mutations cluster at Xp11 (Ropers et al, Trends Genet. 19:316,2003; see also abstract Jensen et al, this meeting). Here we report that mutations in the gene coding for the polyglutamine tract binding protein 1 (PQBP-1), which maps to Xp11.2, lead to the previously described Sutherland-Haan and Hamel syndromes, characterized by mental retardation, microcephaly, short stature, spastic paraplegia as well as different midline defects, or to non-syndromic XLMR. PQBP-1 binds to a wide variety of transcription factors and has been implicated in the pathogenesis of polyglutamine expansion diseases such as cerebellar ataxias and Huntington chorea. We found 5 frameshift mutations in 27 unrelated families with linkage intervals overlapping the proximal Xp. All mutations disrupt the polyglutamine-binding PRD domain and result in truncated proteins lacking a nuclear localisation signal as well as an evolutionarily conserved C-terminal domain, which binds to a component of the spliceosome. Functional studies strongly suggest that PQBP-1 mutations affect basal transcription. Our findings point toward PQBP-1 as an important player in syndromic and non-syndromic XLMR and shed more light on the pathogenesis of this frequent disorder.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

X-Linked Spermine Synthase Gene (SMS) Defect: The First Polyamine Deficiency Syndrome. *C.E. Schwartz[1], A.L. Cason[1], Y. Ikeguchi[2], C. Skinner[1], T.C. Wood[1], D.E. McCloskey[2], K.R. Holden[1], H.A. Lubs[3], F. Martinez[4], R.J. Simensen[1], R.E. Stevenson[1], A.E. Pegg[2].* 1) Greenwood Genetic Ctr, Greenwood, SC; 2) Dept. of Cellular and Molecular Physiology, Penn State Univ, Hershey, PA; 3) Univ of Miami, School of Medicine, Miami, FL; 4) Unidad de Gentica, Hospital Univ La Fe, Valencia, Spain.

Polyamines are essential for normal cell growth and differentiation. However, little is known about the specific cellular functions of these compounds and despite extensive laboratory investigations, there are no known heritable conditions in humans in which polyamine synthesis is perturbed. Studies of fibroblasts from the gyro (Gy) mutant mouse, in which spermine was completely absent due to the disruption of spermine synthase (Sms), an X-linked gene, found no alteration in growth rate or overt morphological changes. In contrast, the Gy mouse exhibits neurological abnormalities. We report that a splice mutation in spermine synthase is responsible for the Snyder-Robinson syndrome (SRS; OMIM 309583), an X-linked mental retardation (XLMR) disorder. The affected males have mild-to-moderate MR, childhood hypotonia, facial asymmetry, thin habitus, osteoporosis, kyphoscoliosis, decreased activity of spermine synthase, low levels of intracellular spermine and elevated spermidine/spermine ratios. These findings represent the first recognition of a role for spermine in cognitive function and likely reflect the importance of spermine's ability to function as an intrinsic gateway molecule for rectifying K+ channels. The ability to measure activity of spermine synthase and the spermidine/spermine ratio in white cells makes it possible to diagnose SMS deficiency in male patients with mental retardation of unknown etiology. In vitro studies, using patient cell lines, showed that the addition of exogenous spermine, with or without the drug DFMO, increases spermine levels and restores the spermidine/spermine ratio. Similar studies using the Gy mouse and a diet supplemented with spermine with or without DFMO are presently underway. Our findings are significant relative to the possibility of treatment for this metabolic condition.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

How does the mode of inheritance of a genetic condition influence families? *C.A. James[1], J.A. Winkelstein[1], N.A. Holtzman[1], D.W. Hadley[2].* 1) Johns Hopkins Medical Institutions, Baltimore, MD; 2) NHGRI, National Institutes of Health, Bethesda, MD.

While the mode of inheritance of a genetic condition has long been considered to have not only medical but also psychosocial consequences for families, this supposition has never been tested. The X-linked (XL) and autosomal recessive (AR) forms of chronic granulomatous disease (CGD) provide a model for investigating the effect of mode of inheritance while controlling for differences in phenotype. We surveyed 112 members of 51 families (59% response) with CGD to determine the influence of mode of inheritance on parents, siblings, and patients (1) understanding of inheritance and reproductive risk, (2) concern about risk to future family members, (3) feelings of guilt and blame, and (4) feelings of stigmatization. Ninety-six members of 51 families (49% response) with Duchenne/Becker muscular dystrophy (XL) and spinal muscular atrophy types II /III (AR) were also studied. Members of XL families understood the mode of inheritance ($p<0.001$) and their reproductive risks ($p<0.01$) better than AR family-members. XL mothers worried about risks to future generations more than AR mothers, but other AR family members were as worried as their XL counterparts. Most XL mothers felt guilty about, and blamed themselves for, their childs disease. XL mothers were more likely to feel guilty ($p<0.01$) and blame themselves ($p<0.001$) than were AR mothers or fathers of either mode of inheritance ($p<0.001$). Conversely, XL fathers were more likely than XL mothers ($p<0.001$) or AR fathers ($p<0.05$) to admit to blaming their childs other biological parent for the condition. XL family members were also more likely to consider learning one was a carrier stigmatizing ($p<0.05$). This study has implications for improving genetic counseling for families with childhood-onset XL and AR conditions. Counseling should assist families in understanding, and coping with, both the medical and psychosocial implications of the inheritance pattern. Particular attention should be given to 1) issues of guilt and blame in XL families and 2) understanding of reproductive risks in AR families.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 15 from 2003 ASHG Annual Meeting

Classification of cardiovascular malformations in the National Birth Defects Prevention Study. *A. Lin[1], L. Botto[2], S. Ghaffar[3], C. Cosper[2], A. Correa[2], and the Natl Birth Defects Prevention Study.* 1) Genetics & Teratology, Ped Service, MGH, Boston, MA; 2) Natl Ctr Birth Defects & Devel Disabil, Ctrs for Disease Control and Prevention, Atlanta, GA; 3) Cardiology, Arkansas Children's Hospital, Little Rock, AR.

The causes of most cardiovascular malformations (CVMs) are unknown. To identify their environmental and genetic risk factors, CVMs have been included in the National Birth Defects Prevention Study (NBDPS), a multi-center population-based case-control study of birth defects. Studying CVMs has been a persistent challenge because of the number of individual CVMs and frequent occurrence as multiple defects. To optimally evaluate cases as meaningful groups, studies have used systems based purely on 1) embryologic mechanisms or entirely on 2) individual heart defects (eg. ICD codes). The former presumes pathogenetic mechanism; the latter can be unwieldy and dilute meaningful groupings. We report on a computer assisted approach to classify CVM cases in the NBDPS which includes a framework using elements of both systems. METHODS: Prior to the actual classification, we examined the patterns of association among CVMs in over 2,000 CVM cases to create the main classification entries. In addition, we devised an additional descriptor of complexity categories: Simple (one or essentially one defect, eg. ASD secundum), Association (2 defects, not reducible into a simple defect, eg. ASD secundum and coarctation), Complex (3 or more defects, eg. ASD secundum, coarctation and single ventricle). From 10/97-10/02, 6271 cases with a CVM had been registered by participating NBDPS Centers. We reviewed and classified 945 with at least 1 conotruncal diagnosis. RESULTS: Classification of CVMs (and not other defects in the same child) rated 715 (76%) as Simple, 101 (11%) as an Association, 109 (11%) as Complex and 20 (2%) as Unclassified, Other or Unclear. CONCLUSIONS: We applied a computer assisted approach for classification designed to integrate clinical information. Since most conotruncal CVMs could be classified as "Simple" CVMs, such "streamlining" should promote homogeneous study groups for the future risk factor studies.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 16 from 2003 ASHG Annual Meeting

Deletion of connexin 40 (GJA5) in association with congenital heart defects. *M.J. Somerville, M. Hicks, J. Christiansen, K. Sprysak, R. Tomaszewski, B.G. Elyas, S.M. Hasse, A.Y. Ng, M. Lilley, L.M. Vicen-Wyhony.* Dept Medical Genetics, Univ Alberta, Edmonton, AB, Canada.

Expression of the cardiac gap junction protein, connexin 40 (Cx40) is primarily restricted to the conduction system and atria. Reduced expression of Cx40 has been found in association with impaired conduction of dilatory signals along arterioles, mitral valvular disease, and chronic atrial fibrillation. Previous attempts at identifying point mutations in the gene coding for Cx40 (GJA5) in cases of familial atrial fibrillation syndrome have proven unsuccessful. We screened 466 unrelated DiGeorge and William syndrome-negative congenital heart defect cases for deletions of GJA5, in order to determine whether hemizygosity for this gene may be associated with a cardiac phenotype. Dosage determination was obtained by real-time quantitative PCR spanning a portion of GJA5 coding exon 2, and confirmed by semi-quantitative PCR using a primer array across the open reading frame. All apparent positive cases were sequenced to determine whether any possible PCR-refractory point mutation was present. In total, 3 cases were found to carry deletions of the entire GJA5 coding region (3/466, freq. 0.006). No point mutations were detected. Of 330 unrelated normal controls screened, none carried the GJA5 deletion ($p < 0.12$). We hypothesize that hemizygosity for GJA5 is associated with a particular subset of cardiac defects. All 3 cases had structural cardiac malformations in common without any consistent dysmorphic features. The cardiac defects caused by a deletion of this gene may be restricted to abnormal cell-to-cell communication and alteration in the electrophysiologic properties of the atrium, or may present clinically within the spectrum of other known conditions. For example, animal models of Holt-Oram syndrome have shown reduced expression of Cx40, resulting from TBX5 haploinsufficiency. Further work is underway to determine the extent of the genomic deletion in the 3 cases detected in the present study, and to examine the nature of the cardiac defects relative to those of the non-deleted cases.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 17 from 2003 ASHG Annual Meeting

***Quaking*, an RNA binding protein required for proper myelination, is also essential for cardiovascular development.** *J.L. Northrop[1], L. Pool[1], L. Lai[2], K.K. Hirschi[2], M.E. Lane[3], M.J. Justice[1].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Molecular and Cellular Biology, and Pediatrics, Baylor College of Medicine, Houston, TX; 3) Biochemistry and Cell Biology, Rice University, Houston, TX.

One out of every 150 infants in the United States is born with a significant congenital heart defect. Identifying the role of genes essential to cardiovascular development in vertebrate model organisms provides a window of insight into mechanisms of congenital cardiac defects in humans. We have recently determined that *quaking*, an RNA binding protein, has a previously unsuspected and essential role in cardiovascular development. The *quaking* locus was initially identified in 1964 from a spontaneously occurring mouse mutation that resulted in seizure activity and shaking due to a disruption in myelination. An allelic series of point mutations in the mouse *quaking* gene produce defects that cause embryos to die around E10.5, demonstrating that *quaking* has a critical role in development prior to the onset of myelination. Our previous studies show that *quaking* is expressed in the mouse yolk sac endoderm; adjacent to the mesoderm-derived developing blood islands where differentiation of blood and endothelial cells first occurs. In addition, vessels within the mouse embryo proper exhibit similar defects in endothelial cell maturation and smooth muscle cell recruitment. *Quaking* is also highly expressed in the developing mouse and zebrafish heart. Our current studies delineate its role in early cardiovascular development using both mice and zebrafish as model organisms. Inhibition of the zebrafish *quaking* homolog (*Zqk*) by injection of an antisense morpholino oligonucleotide results in disruption of blood circulation, impairment of truncal vascular development, and abnormal cardiac function in the developing zebrafish. While previous studies of *quaking* have shown that its expression is essential for postnatal myelination, these results demonstrate a novel and critical role for *quaking* in cardiovascular development.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Perturbation of alternative splicing in a novel cardiocutaneous progeria syndrome caused by mutation in lamin A/C. *D.P. Judge[1], C.M.J. ap Rhys[1], P. Guerrerio[1], J. Geubtner[1], J. Zhang[1], A. Cheng[1], H.C. Dietz[1,2]*. 1) Johns Hopkins Univ Sch Med, Baltimore, MD; 2) HHMI.

We studied a family segregating a novel, dominant, cardiocutaneous progeria syndrome (CCPS) affecting 6 individuals in 4 generations. Manifestations involve premature aging phenotypes in late adolescence including graying of hair, loss of fat, wrinkled skin, dysfunction of cardiac valves, and aggressive atherosclerosis involving the coronary, cerebral, and peripheral arterial systems. The average age at death is 36.5 years. Additional manifestations include skin malignancy and nephrosclerosis. Genome-wide linkage analysis revealed a suggestive peak at 1q21.2. The recent demonstration of mutations in *LMNA*, that maps to 1q21.2 and encodes lamins A and C, in Hutchinson-Gilford progeria syndrome (HGPS), prompted mutational screening. We identified a novel *LMNA* mutation that segregates with disease, substitutes an evolutionarily conserved residue, and was absent in a large control population. *LMNA* mutations have been associated with a remarkable diversity of phenotypes including multiple muscular dystrophies, partial lipodystrophy, mandibuloacral dysplasia, dilated cardiomyopathy, and HGPS. Distinguishing features of CCPS include the lack of muscular and bony disease, alopecia, characteristic facies, childhood mortality (mean 13.4yrs) and the common *de novo* mutation associated with HGPS. Phenotypic diversity is difficult to reconcile on the basis of simple phenotype-genotype correlations. Lamins localize to the inner nuclear membrane and to nucleoplasmic concentrations of splicing factors. As in other laminopathies, CCPS cells show abnormalities of nuclear morphology. We reasoned that assessment of nonsense-mediated mRNA decay (NMD) and nonsense-mediated altered splicing (NAS) would serve as a marker of adequacy of many cellular events. CCPS cells show normal NMD, documenting intact constitutive splicing, transcript trafficking, and translational efficiency. However, CCPS cells show a profound deficiency in NAS. These data further document the relevance of lamins to aging and offer the hypothesis that perturbations of alternative splicing contribute to disease pathogenesis.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 19 from 2003 ASHG Annual Meeting

Screening and functional analysis of *ZIC3* mutations in heterotaxy and related congenital heart defects. *S.M. Ware*[1], *J. Peng*[1], *L. Zhu*[1], *S. Fernbach*[1], *S. Colicos*[1], *B. Casey*[2], *J.A. Towbin*[1,3], *J.W. Belmont*[1]. 1) Dept Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Children's & Women's Health Centre, Vancouver, BC; 3) Dept of Pediatrics (Cardiology), Baylor College of Medicine, Houston, TX.

Mutations in the zinc finger transcription factor ZIC3 cause X-linked heterotaxy (MIM 306955) and have also been identified in patients with isolated congenital heart disease (CHD). In order to determine the relative contribution of *ZIC3* mutations to both heterotaxy and isolated CHD we screened the coding region of *ZIC3* in 195 unrelated patients, including 45 patients with classic heterotaxy, 106 patients with heart defects characteristic of heterotaxy, and 10 patients with situs inversus. Five novel *ZIC3* mutations in three kindreds and two sporadic cases were identified. None of these alleles were found in 200 ethnically-matched control samples. Based on these analyses, the phenotypic spectrum of *ZIC3* mutations should be expanded to include affected females and non-classic heterotaxic CHD. This screening of a cohort of sporadic heterotaxy patients indicates that *ZIC3* mutations account for approximately 1% of affected individuals. Missense and nonsense mutations were found both within the highly conserved zinc finger binding domain as well as in the N-terminal protein domain. Functional analysis of all currently known *ZIC3* mutations indicates a loss of luciferase reporter gene transactivation both with mutations in the putative DNA binding domain of the zinc finger region as well as with mutations in the N-terminal domain. Surprisingly, subcellular localization studies demonstrate aberrant cytoplasmic localization resulting from mutations between amino acids 253-325 of the ZIC3 protein, indicating that the pathogenesis of a subset of *ZIC3* mutations results from failure of the mutant protein to localize to the nucleus. These results further expand the phenotypic and genotypic spectrum of *ZIC3* mutations and provide initial mechanistic insight into their functional consequences.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 20 from 2003 ASHG Annual Meeting

Identification of New Genes Differentially Expressed in Coronary Artery Disease by Expression Profiling. *S.R. Archacki, G. Angheloiu, X. Tian, C. Moravec, E. Topol, Q. Wang.* Molecular Cardiology, Cleveland Clinic Foundation, Cleveland, OH.

Genetic factors increase the risk for coronary artery disease (CAD). To date, a limited number of genes that potentially contribute to the genesis of CAD have been identified. In this study, we have performed a large-scale gene expression analysis of ~12,000 human genes in nine severely atherosclerotic and six non-atherosclerotic human coronary arteries using oligonucleotide microarrays. Fifty-six genes were differential expressed. The expression of 55 genes were increased in CAD whereas only one gene, glutathione-S-transferase encoding a reducing agent, showed down-regulated expression. Selected genes were validated by quantitative RT-PCR analysis. Furthermore, we show an increased expression of four proteins in atherosclerotic arteries. These include: MM-9, PIM-2 oncogene, osteopontin, and ICAM-2. The associations of 49 genes with CAD appear to be novel and they include genes encoding ICAM-2, PIM-2, ECGF1, fusin, B cell activator (BL34, GOS8), Rho GTPase activating protein-4, retinoic acid receptor responder, b2-arrestin, membrane aminopeptidase, cathepsin K and H, MIR-7, TNF a-induced protein 2 (B94), and flavocytochrome 558. In conclusion, we have identified 56 genes which are expressed in human coronary arteries with atherosclerosis, and 49 of them may represent new genes linked to CAD and may offer insight to define novel molecular pathways and genes involved in the generation of CAD.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Diet control strengthens the effect of the lipoprotein lipase (LPL) gene on lipids. *X. Guo[1], K.D. Taylor[1], M.O. Goodarzi[1], R.M. Krauss[2], J.I. Rotter[1]*. 1) Cedars-Sinai Medical Center & UCLA, Los Angeles, CA; 2) Lawrence Berkeley National Laboratory, UC Berkeley, CA.

Lipid and lipoprotein traits have been shown to aggregate in families, but studies of genetic linkage and association of these traits have yielded inconsistent results. In a previous study we have shown that heritability estimates for LDL-C and HDL-C, after administration of a high fat (HF) diet, were greater than estimates on an uncontrolled diet, suggesting that the use of defined dietary conditions to control for gene-diet interactions may improve the power to identify specific genes affecting plasma lipoprotein metabolism. Lipoprotein lipase (LPL) controls the delivery of fatty acids to muscle and adipose tissue by hydrolyzing circulating triglycerides and so is a candidate gene for lipid and lipoprotein traits. In a study aimed at identifying the genetic influences on the responses of lipid and lipoprotein traits to a low-fat, high carbohydrate diet, 317 healthy male sibs in 141 families were placed for 4 weeks each on iso-caloric high-fat (41% energy, HF) and low-fat (21% energy, LF) diets. Lipid and lipoprotein traits were measured at baseline (usual diet) and after each of the experimental diets. Sibs and their parents (~600 individuals total) were genotyped for six polymorphisms in the 3-end of the LPL gene (7315, 8292, 8393 (HindIII), 8852, 9040 (Ser447Stop), and 9712) using TaqMan MGB technology. Using the pedigree structure and genotype data of all individuals in each pedigree, haplotypes were reconstructed using the maximum likelihood method as implemented in Simwalk2. Six LPL haplotypes account for over 99% of all haplotypes. In the subsequent association analysis using QTDT, we found that haplotype 5 is significantly associated with triglycerides under both high-fat and low-fat diets (p=0.0084, and 0.047), but not with baseline triglycerides. We also detected an association of haplotype 2 with Lp(a) under both high-fat and low-fat diets (p=0.012, and 0.011, respectively), but not with baseline Lp(a). These results support the advantage of intervening on environmental factors in a genetic study.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 22 from 2003 ASHG Annual Meeting

Identification of the genetic basis of individual differences in human serum high-density lipoprotein cholesterol (HDL-C) concentration. *K.A. Frazer[1], A.B. Seymour[2], J.F. Thompson[2], D.A. Hinds[1], P. Banerjee[2], K.L. Durham[2], D.G. Ballinger[1], D.R. Cox[1], P.M. Milos[2].* 1) Perlegen Sciences, Mountain View, CA; 2) Pfizer, Groton, CT.

Low levels of human serum high-density lipoprotein cholesterol (HDL-C) are strongly correlated with cardiovascular disease. To determine the genetic basis of individual differences in serum HDL-C concentration, we have carried out a genetic association study using 7283 SNPs surrounding 72 genes with plausible roles in influencing serum HDL-C levels. These SNPs represent 955 haplotype blocks, spanning 17.1 megabases of genomic DNA, at an average density of one SNP every 2.3 kb. Genomic DNA from 345 individuals with low serum HDL-C levels, and 321 individuals with high serum HDL-C levels was analyzed to identify SNPs with large allele frequency differences between the two groups. Allele frequency differences were initially estimated using four DNA pools, containing equal contributions of genomic DNA from each of 173 individuals with low HDL-C (pool A), 172 individuals with low HDLC (pool B), 161 individuals with high HDL-C (pool C), and 160 individuals with high HDL-C (pool D). Allele frequency determination of each of 7283 SNPs was carried out in triplicate in each pool by PCR amplification followed by hybridization to a high-density oligonucleotide array genotyping platform. 6611 of the 7283 SNPs (91%) passed data quality filters and resulted in low standard errors for replicate allele frequency determinations. 284 SNPs (4% of the total) with the largest allele frequency differences averaged across subpools were chosen for further confirmation by genotyping using individual, non-pooled DNA samples. The results indicate that quantitative SNP allele frequency determination followed by genotype determination on individual samples using high density oligonucleotide arrays provides a powerful method for identifying the genetic basis of complex human traits.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 23 from 2003 ASHG Annual Meeting

Molecular genetic screening of -myosin heavy chain in dilated and hypertrophic cardiomyopathy. *E. Carniel*[1], *M.R.G. Taylor*[1], *P.R. Fain*[1], *A. Di Lenarda*[2], *G. Sinagra*[2], *J. Lascor*[3], *L. Ku*[1], *J. Feiger*[1], *D. Slavov*[1], *X. Zhu*[1], *D. Dao*[1], *D.A. Ferguson*[1], *L. Mestroni*[1]. 1) University of Colorado Cardiovascular Institute, Denver, CO; 2) Division of Cardiology, University of Trieste, Trieste, Italy; 3) Division of Cardiology, The Children's Hospital, Denver, CO.

Myosin heavy chains are sarcomeric proteins expressed in the human ventricular myocardium in two different isoforms: and encoded as MYH6 and MYH7 respectively. The isoform has a higher ATPase activity than the isoform and leads to a more rapid contraction of the cardiac fibers. In failing hearts a down regulation of the isoform and an up regulation of the isoform have been shown to correlate with the systolic dysfunction observed in heart failure. Mutations in MYH7 can cause dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM). We tested the hypothesis that MYH6 mutations can cause the same diseases. One hundred and two subjects, 93 with DCM (27 sporadic; 66 familial, belonging to 45 families) and 9 with HCM (belonging to 8 families), were screened for MYH6 mutations using denaturing high performance liquid chromatography and sequence analysis. Variants were screened in a control population of 150 healthy subjects ethnically matched. Protein secondary structure was predicted with GOR4 and nnpredict software. Four putative MYH6 mutations have been discovered in 4 families: 3 with sporadic DCM (P830L, A1004S, E1457K) and 1 with HCM (Q1065H). The A1004S mutation changes an alanine (nonpolar residue) to a serine (polar residue). The Q1065H mutation changes a glutamine (uncharged residue) to a histidine (charged, residue). The P830L mutation changes a proline to a leucine and is predicted to alter the secondary structure of the light chain binding domain. The E1457K mutation changes a glutamic acid (acidic residue) to a lysine (basic residue) at codon 1457 and may alter the alpha helix of the rod domain. All mutations are absent in 150 normal controls (and in over 500 chromosomes) and occur in residues conserved across different species. Two novel polymorphisms (G56R, E1295Q) did not cosegregate with the disease within the families. These data suggest that mutations in MYH6 may cause DCM or HCM.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 24 from 2003 ASHG Annual Meeting

Premature myocardial infarction novel susceptibility loci identified by genome-wide linkage analysis. *Q. Wang*[1, 2, 6], *S.-Q. Rao*[1, 2, 6], *G.-Q. Shen*[1, 2], *L. Li*[1, 2], *K. Newby*[4], *W.J. Roger*[5], *R. Cannata*[1], *E. Zirzow*[1], *R.C. Elston*[3], *E.J. Topol*[1, 2]. 1) Ctr for Cardiovascular Genetics, Dept. of Cardiovascular Medicine, Cleveland Clinic Foundation, Cleveland, OH; 2) Ctr for Molecular Genetics, ND40, Cleveland Clinic Foundation, Cleveland, OH; 3) Dept. of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH; 4) Duke Clinical Research Institute, Duke University Medical Center, Durham, NC; 5) Dept. of Medicine, the University of Alabama Medical Center, Birmingham, AL; 6) These authors contributed equally to this work.

Atherosclerotic coronary artery disease (CAD) and acute myocardial infarction (MI), the most important cause of death and disability in the Western world, are thought to have a polygenic basis with a complex interaction with environmental factors such as lifestyle. We recruited 428 multiplex families with premature CAD and MI consisting of 2030 individuals - 712 with MI, 974 with CAD and the age of onset was 44.4 9.7 yrs. Genotyping was performed at the NHLBI mammalian facility using 408 markers that span the entire human genome every 10 cM. Linkage analysis was performed with the modified Haseman-Elston regression models and SIBPAL program. Three genome wide scans were conducted for single point, multipoint, and multipoint Caucasian only analysis (92% of cohort). Of eight novel susceptibility loci detected for MI, the most significant linkage was found to chromosome l (multipoint allele-sharing P values of -log(P)>12, log of the odds ratio (LOD) score of 11.98); linkages to chromosomes 2, 4, 5, 7, 12, 13, 14 meet the criteria for genome-wide significance (-log(P)= 4.66 or LOD score of 3.6). For the less restrictive phenotype of CAD, no genetic loci were detected. Our data suggest that CAD and MI are genetically distinct disorders that may not share all susceptibility genes. This study thus identifies novel genetic susceptibility loci for MI, and provides a framework for the ultimate cloning of genes for premature, familial MI.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 25 from 2003 ASHG Annual Meeting

A translation-independent role for TCR mRNA in fetal thymic development. *P.A. Frischmeyer-Guerrerio, R.A. Montgomery, S.K. Cooke, D.S. Warren, C.J. Sonnenday, A.L. Guerrerio, H.C. Dietz.* HHMI, Johns Hopkin. Sch. Med., Balt, MD.

Nonsense-mediated mRNA decay (NMD) is the highly conserved process by which eukaryotic cells recognize and degrade transcripts harboring premature termination codons. We previously reported that transgenic mice expressing a dominant-negative trans-effector of NMD show relative loss of function and impaired T cell maturation. This correlates temporally with stabilization of nonsense TCR transcripts derived from alleles that underwent nonproductive (out-of-frame) rearrangement, a high-frequency physiologic event during the generation of immunologic diversity. The mutant thymus showed a specific deficiency in the frequency of V-to-DJ recombinations, which are subject to inhibition by a prior productive recombination event (allelic exclusion), but a normal frequency of D-J recombinations (which are immune to allelic exclusion). These data suggested that either stabilized TCR nonsense transcripts or the truncated proteins they encode may be sufficient to initiate inappropriate allelic exclusion. We created transgenic mice that allow us to distinguish between these possibilities. The LVDJ line, expressing a truncated TCR peptide (the predicted product of a typical stabilized nonsense TCR mRNA), showed no phenotypic abnormalities. A second line of transgenic mice (TCRRNA) expresses full-length stable TCR message that fails to make TCR protein due to site-directed mutations that place both alternative start codons out-of-frame. Remarkably, NMD-proficient TCRRNA mice fully recapitulate all abnormalities observed in NMD-deficient animals including a developmentally-restricted reduction in CD4/CD8 double positivity with an increase in double negative cells, failure to downregulate CD25, and reduced expression of TCR. This supports a model in which allelic exclusion is initiated by stable TCR message and consolidated by pre-TCR signaling. These data support two novel and important paradigms. First, NMD has been functionally incorporated during the evolution of essential developmental and homeostatic processes in higher eukaryotes. Second, selected mRNAs can support essential functions independent of their contribution to protein production.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Frizzled 9 knock-out mice have intrinsic defect in B cell development and function. *H. Kwan, E. Ranheim, Y. Wang, T. Reya, I. Weissman, U. Francke.* Departments of Genetics and Pathology, Stanford University, Stanford CA.

Frizzled 9 (FZD9) is located in 7q11.23 and is deleted in Williams-Beuren syndrome. It encodes one of the frizzled receptors, 7-transmembrane domain proteins that, upon binding to Wnt proteins, function in beta-catenin/ T cell factor (TCF) signaling, a p athway involved in neural development, cell polarity generation, cell fate specification, tumorigenesis and self renewal ability of stem cells. To investigate a possible contribution of FZD9 to the WBS phenotype, Fzd9 knockout mice were created. Heterozygotes were normal, but 40% of Fzd9-/- homozygotes died by six months of age and fewer than 5 % lived longer than 18 months. At six months of age, a two fold increase of spleen weight and a three-fold decrease of thymus weight were found as compared to wildtype control. Lymph nodes were enlarged with normal T and B cell percentages, but increased numbers of plasma cells. FACS analyses revealed a severe depletion of pro-B cells (CD43+B220+) in the bone marrow, especially in the B and C Hardy subsets where heavy chain is expressed and the cells are undergoing clonal expansion prior to light chain rearrangement. Bone marrow stem cell competition reconstitution experiments suggested that this pro-B cell defect is intrinsic to the hematopoietic system. Interestingly, mature B cells are hypo-responsive to mitogenic stimulation. These results suggest a role for frizzled 9 signaling in lymphoid development, possibly at a stage where B-cells undergo self-renewal prior to further differentiation.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

A signature of balancing selection in the human CD4 gene. *S. Prahalad*[1], *S. Wooding*[2], *D. Dunn*[2], *R. Weiss*[2], *L.B. Jorde*[2], *M. Bamshad*[1,2]. 1) Pediatrics, U of Utah, Salt Lake City, UT; 2) Human Genetics, U of Utah, SLC, UT.

CD4 encodes a T-cell surface receptor that plays essential roles in antigen recognition, interactions with antigen-presenting cells, and T-cell activation. CD4 is also exploited by pathogens such as HIV-1 to enter T-cells and establish infection. CD4 is therefore critical for immune system function, yet is a source of vulnerability to pathogens. Thus, *CD4* is under both strict functional constraints and pressure to evolve defenses against pathogens. To investigate the effects of natural selection and population history on patterns of variation in CD4, we examined DNA sequence variation in 168 chromosomes collected from African, Asian, and European populations. Patterns of variation in *CD4* did not deviate significantly from neutral expectations, assuming no recombination. However, exons 1-3 and exons 4-10 of CD4 are separated by ~13 kb, and a scatterplot of pairwise linkage disequilibrium (LD) estimates vs. physical distance revealed that LD dropped substantially between these two regions. Thus, each region is on a different haplotype block. We re-analyzed each block separately. The pattern of variation in block 2 was consistent with neutrality, whereas block 1 was characterized by an excess of diversity, a positive Tajimas D (D=2.14; $p<0.02$), and 2 high-frequency haplotypes separated by more mutation steps (4) than expected under neutrality. Because population growth and structure are expected to influence each of these blocks similarly, the different patterns of variation observed are likely the result of different selective pressures. All of the polymorphisms in the coding exons of block 1 are silent, suggesting that selection might be acting instead on polymorphisms in non-coding regions that influence *CD4* regulation. This recapitulates the pattern observed in the 5 *cis*-regulatory region of *CC chemokine receptor 5*, another T-cell receptor exploited by HIV-1. Invading a host via proteins that are functionally constrained against variation might be a particularly effective strategy for pathogens. Our results suggest that, in response, changes in host genes that alter the regulation of these proteins might have become a preferred counter-measure.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 28 from 2003 ASHG Annual Meeting

Developing population based newborn screening for severe combined immunodeficiency. *K. Chan*[1,2], *J.M. Puck*[1].
1) Genet & Mol Biol Branch, NHGRI, NIH, Bethesda, MD; 2) Yale Epi and Pub Health, New Haven, CT.

Severe combined immunodeficiency (SCID), a syndrome of unknown incidence (estimated 1/50,000-100,000 births), leads to failure to thrive, severe infections, and death in infancy unless immune reconstitution is provided. Treatment by bone marrow transplantation (BMT) within 3.5 months may rescue >95% of SCID infants, while those diagnosed later have decreased survival (Myers 2002; Buckley 1999). Currently no cost-effective screening method exists for SCID, which is caused by diverse mutations in many genes, including IL2RG, ADA, JAK3, IL7RA, RAG1/2, ARTEMIS and CD45. However, all SCID patients have few or no T cells. We thus proposed to detect SCID by the absence of T cell receptor (TCR) excision circles (TRECs), byproducts of TCR gene rearrangement that are found in recently formed T cells and are most abundant in normal newborns. A single, circular junction fragment formed by excision of the TCRD gene from the TCRAD locus occurs in 70% of alpha-beta TCR T cells and is detectable by PCR (Douek 1998). We developed a real-time PCR assay for TRECs using DNA from dried blood on Guthrie cards routinely collected for newborn screening. We made DNA from two 3-mm discs punched from dried spots of anticoagulated normal cord blood, adult control blood, blood from which T cells were depleted in the laboratory, and blood from newly diagnosed SCID infants referred to our lab for mutation detection. DNA was isolated and assayed by real-time PCR for copy number of TRECs. Normal cord blood (n=11) had a mean of 752 TRECs; adult blood (n=6) 94 (lower, as expected); T-cell-depleted blood (n=3) undetectable to <49; and SCID patient blood (n=2) undetectable to <30. Anonymous, outdated Guthrie cards that had been stored dry at -20 C for >1 year (provided by the Maryland State Health Dept) had an average of 646 TRECs. Thus TRECs represent a stable analyte that can be measured in actual Guthrie cards and can distinguish samples with normal numbers of T cells from samples lacking T cells. While more samples will be needed to establish ranges for optimal sensitivity and specificity, our assay could evolve into an inexpensive, rapid, large-scale newborn screening test for SCID.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 29 from 2003 ASHG Annual Meeting

Claudin-1 gene mutation in Neonatal chthyosis-Sclerosing cholangitis. *S. Hadj-Rabia[1,2], L. Baala[1], P. Vabres[3], M. Fabre[4], E. Jacquemin[5], S. Lyonnet[1], Y. de Prost[2], M. Hadchouel[5], A. Munnich[1], A. Smahi[1]*. 1) Dept Genetics, Hosp Necker, Paris, France; 2) Dept Dermatology,Hosp Necker, Paris, France; 3) Dept Dermatology, CHU, Poitiers, France; 4) Dept of pathology, CHU, KremlinBictre, France; 5) INSERM U-347, CHU , Kremlin Bictre, France.

Neonatal Ichthyosis Sclerosing Cholangitis (NISCH, MIM607626) is a rare autosomal recessive disease characterized by ichthyosis and inflammation and obliterative fibrosis of the hepatic bile ducts, leading to liver failure. We mapped the disease gene to a 16.2 cM interval on chromosome 3q27-q28 by homozygosity mapping in two unrelated inbred Moroccan kindred. The four affected children shared a 9.5-cM common haplotype supporting a founder effect in NISCH. Ultrastructural analysis of the skin showed anchoring plaques of desmosomes suggesting involvement of a cohesive protein. Therefore, claudin-1 (CLDN-1) was regarded as a strong candidate gene based on its mapping to the minimum interval and on the expression pattern of the mouse orthologue in tight junctions (TJ) of tissues involved in the NISCH. The four patients shared a homozygous deletion causing a frame shift and a premature translation termination in the CLDN-1 gene. Consistently, Western blot showed absence of CLDN-1 in liver and cultured skin fibroblasts of patients while immunostaining showed a specific staining in bile ducts and canaliculi of normal liver . In association with occludin, CLDN-1 forms the backbone of integral membrane proteins of TJ which maintain cell polarity and control paracellular permeability in high resistance epithelia. CLDN-1 KO mice died from early dehydratation: dry ichthyosic skin could be ascribed to impaired ionic selectivity in NISCH. In the liver, absence of CLDN-1 may either lead to abnormal electrolyte bile composition and cholestasis or to increased paracellular permeability between biliary epithelial cells. This is the first report of the pleitropic effect of a claudin gene mutation affecting skin and liver. The identification of CLDN-1 mutation in the NISCH syndrome emphasizes the crucial role of claudins, and more generally of TJ proteins, in inherited disorders involving epithelial tissues.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 30 from 2003 ASHG Annual Meeting

Hyper-IgD and periodic fever syndrome: a role for the isoprenoid biosynthetic pathway in regulation of fever and inflammation. *H.R. Waterham[1], M. Schneiders[1], J. Koster[1], S.M. Houten[1], S. Mandey[1], J. Frenkel[2], R.J.A. Wanders[1].* 1) Lab. Genetic Metabolic Diseases, Academic Medical Center, Amsterdam, The Netherlands; 2) General Pediatrics, University Medical Center, Utrecht, The Netherlands.

Hyper-IgD and periodic fever syndrome (HIDS) and mevalonic aciduria (MA) are autosomal recessive disorders characterized by recurrent episodes of high fever and generalized inflammation. Both syndromes are caused by specific mutations in the mevalonate kinase (MK) gene (MVK), resulting in deficient MK activities. MK is the first enzyme following the highly regulated and rate-limiting HMG-CoA reductase (HMGR) in the isoprenoid biosynthetic pathway. We report here the mutational spectrum obtained after analysis of over 50 patients with HIDS or MA through sequencing of coding MVK sequences amplified by PCR. Previously, we showed that the predominant HIDS mutation V377I primarily affects MK protein levels and that these levels can be manipulated by culturing patient cells at different temperatures. At 30C HIDS cells displayed substantially higher MK activities than at 37C due to increased MK protein levels, while at 40C, MK levels and activity decreased further. This decrease triggered a compensatory increase in HMGR activity indicating that MK becomes progressively rate-limiting. Our recent data indicate that the link between MK deficiency and inflammatory response may be related to a (temporary) shortage of geranylgeranyl moieties due to a reduced flux through the pathway at elevated temperatures. In an attempt to increase the flux we have studied the effect of adding simvastatin (HMGR inhibitor) and zaragosic acid (squalene synthase inhibitor) to HIDS cells. We found that both drugs cause a significant increase in MVK transcription (lightcycler analysis) and as a consequence result in an elevation of MK levels and activity suggesting that manipulation of the isoprenoid biosynthetic pathway in MK deficient patients may provide a potential therapeutic option.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Identification and Confirmation of SLE Susceptibility Locus (SLEB4) at 12q24. *S.K. Nath, A.I. Quintero-Del-Rio, J. Kilpatrick, J.B. Harley.* Arthritis & Immunology, Oklahoma Medical Res Fndn, Oklahoma City, OK.

Systemic Lupus Erythematosus (SLE), a chronic complex disease, is a prototype for the systemic human autoimmune diseases. Although environmental factors are crucial in triggering SLE, strong genetic basis is well established. SLE shows extreme variation in its incidence pattern in gender as well as ethnic origin. To identify novel susceptibility loci for SLE, we performed a genome-wide scan with 318 markers on 37 Hispanic families. Since the disease model was uncertain, we performed the initial genome scan based on non-parametric penetrance-independent affected-only allele-sharing methods. We have identified the evidence of linkage (Zlr > 2.3, P<0.01) at 12q24, 16q13 and 20p12 by multipoint analysis. The evidence at 12q24 (Zlr=3.01, P<0.001)exceeded the recommended threshold for genome-wide suggestive linkage. The highest peak identified from both two-point and multipoint analyses were at the same position on marker D12S395. To replicate our initial findings, we tested linkage on three adjacent markers (the peak marker and two adjacent markers, each one from either side) used in genome scan, on an independent data set of 147 families. This data set consisted of 92 European-American (EA) and 55 African-American (AA) families. Based on multipoint linkage analysis, the initial linkage signal at 12q24 was replicated in EA (Zlr=2.88, P<0.002) but not in the AA (Zlr=0.39, P<0.34). Therefore, we combined our Hispanic families with EA families (a total of 129 families). The evidence of linkage met the criteria for genome-wide significance (Zlr=4.2, $P<1x10^{-5}$) and the peak was found at D12S395. The corresponding non-parametric LOD was 3.84. The most parsimonious genetic model (using parametric linkage analysis) at 12q24 was a dominant genetic model (LOD=2.54, HLOD=3.40, =0.65). To confirm the linkage at 12q24, we have used another set of 101 independent EA families. This evidence of linkage has confirmed (Zlr=2.71, P< 0.003, HLOD=1.7, =0.65). Therefore, our results have identified, established and confirmed a major susceptibility locus at 12q24 for SLE, especially in families with Hispanic and EA background. We have designated this susceptibility locus as SLEB4.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 32 from 2003 ASHG Annual Meeting

Polymorphisms in the endothelin-2 (EDN2) gene are associated with asthma in U.S. Hispanics and other asthma-related phenotypes in African-Americans and U.S. Caucasians. *M.J. Basehore, T.D. Howard, E.J. Ampleford, W.C. Moore, D.A. Meyers, E.R. Bleecker.* Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC 27157.

Asthma is a common disorder characterized by variable airway obstruction, bronchial hyperresponsiveness (BHR), and acute and chronic bronchial inflammation. Two associated phenotypes are BHR and elevated total serum IgE levels, both of which have a strong genetic component. Previously, we have shown evidence for linkage to 1p32-34 for asthma (LOD=2.92; Xu et. al, 2001) and a significant TDT result for D1S193 (p=0.0002) in 40 Hispanic families. Several candidate genes reside within this region of linkage, including *endothelin-2 (EDN2)*, whose protein product is a potent bronchoconstrictor. Association studies were performed with 12 publicly available *EDN2* polymorphisms in African-American, U.S. Caucasian, and U.S. Hispanic case-control populations. Asthma in the Hispanics was evaluated first since this population had previously demonstrated linkage to 1p32 for this phenotype. Genetic analysis revealed both SNP and haplotype associations with asthma in the Hispanics (116 cases, 130 controls; p values 0.014 to 0.056). Evaluation of related phenotypes resulted in the association of elevated total serum IgE levels in the African-Americans (166 cases, 269 controls, p values 0.008 to 0.014) and FEV_1/FVC, a measure of asthma severity, in the 227 Caucasian asthmatics (p values 0.0048 to 0.052). These data suggest that variations within *EDN2* may be involved in the susceptibility and severity of asthma as well as the severity of airway constriction.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 33 from 2003 ASHG Annual Meeting

Regulatory variant on chromosome 17q24-q25 associated with psoriasis susceptibility. *A.M. Bowcock[1], C. Helms[1], L. Cao[1], J.G. Krueger[2], E.M. Wijsman[3], F. Chamian[2], D. Gordon[4], J. Ott[4], P.-Y. Kwok[5], A. Menter[6].* 1) Department of Genetics, Washington University School of Medicine, St. Louis, MO; 2) Laboratory for Investigative Dermatology, The Rockefeller University, New York, NY; 3) Division of Medical Genetics and Department of Biostatistics, University of Washington, Seattle, WA; 4) Lab of Statistical Genetics, The Rockefeller University, New York, NY; 5) University of California, San Francisco, CA; 6) Department of Internal Medicine, Division of Dermatology, Baylor University Medical Center, Dallas, TX.

Psoriasis is a chronic inflammatory skin disorder of unknown pathogenesis affecting approximately 2% of the Western population (OMIM 177900). Its frequency is elevated in HIV patients and 20%-30% of patients also have psoriatic arthritis. Psoriasis is a complex disease associated with HLA class I alleles although linkage analysis by our group revealed a second psoriasis locus at 17q24-q25 (PSORS2). This was confirmed with independent family sets and other loci have been proposed. We performed family-based association studies with 242 nuclear psoriasis families and 108 markers to localize a psoriasis susceptibility gene on human chromosome 17q24-q25 to a 27 kb region where TDT $p < 0.001$ with multiple markers. Inspection of associated haplotypes revealed five non-coding single nucleotide polymorphisms (SNPs) associated with a gene encoding a protein implicated in diverse aspects of epithelial membrane biology and immune synapse formation in T cells. Within the skin of normal individuals and psoriasis patients its expression is highest in the uppermost stratum Malpighi. One associated SNP ~200bp downstream of the gene lies in a putative binding site for the transcription factor RUNX1. However, the psoriasis-associated allele abolishes this site; a finding that was confirmed with electrophoretic mobility shift assays. This is the second example of loss of a RUNX1 binding site associated with susceptibility to an autoimmune disease. It suggests an important role for RUNX1 in autoimmunity and a cellular mechanism contributing to psoriasis pathogenesis.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Genomewide mapping and analysis of genes for vitiligo and autoimmunity. *R. Spritz[1], K. Gowan[1], G.S. LaBerge[1], A. Alkhateeb[1], D.C. Bennett[2], P. Fain[1].* 1) Human Medical Genetics Prog, Univ Colorado Health Sci Ctr, Denver, CO; 2) Dept Basic Medical Sciences, St. George's Hosp Med Sch, London, UK.

Autoimmune diseases are among the most common diseases, affecting 10-20% of all people. Vitiligo, characterized by white patches of skin and hair, is the most recognizeable autoimmune disease. In a survey of >3500 probands and their famlies, we found that vitiligo, thyroid disease, pernicious anemia, Addison disease, lupus, and type 1.5 diabetes are highly associated, both in probands and in their relatives, indicating that the association among these autoimmune diseases has a genetic basis. By genomewide linkage analysis of 71 Caucasian families we previously mapped a major vitiligo susceptibility locus, *AIS1*, to 1p.

We have now extended this cohort to 94 families and carried out family stratification analysis. *AIS1*-linkage is restricted to larger, 'vertical' families that have other vitiligo-associated autoimmune diseases; *AIS1* is not linked in smaller families. Thus, *AIS1* appears to be an autoimmunity locus, probably involving mainly rare dominant alleles with high penetrance. Two other loci, on 1q and 9q, are only linked to small 'non-autoimmunity' families, and thus may be true vitiligo susceptibility loci, probably involving common, low-penetrance alleles. A number of additional suggestive linkage signals were also detected.

The *AIS1* interval is only 7.4 Mb, and contains relatively few genes. One of these, *FOXD3*, is an attractive biological candidate, located at the precise peak of the *AIS1* LOD. Sequencing in three large 'autoimmunity' vitiligo families identifed rare variants of the *FOXD3* promoter on the vitiligo-linked haplotype in each case. No other genes showed such variants. We are currently analyzing the effects of these *FOXD3* variants on transcription.

Finally, we have also studied vitiligo age-of-onset, which exhibits heritability of 47% in sibs and 90% in concordant MZ twins. We mapped a QTL for vitiligo age-of-onset to chromosome 2p, which may be distinct from vitiligo susceptibility loci *per se*. Our studies have thus begun to dissect the genetic complexity of autoimmunity and vitiligo.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 35 from 2003 ASHG Annual Meeting

Identification of a novel gene causing the pleiotropic features of Nance-Horan Syndrome. *K.P. Burdon*[1,2], *J.D. McKay*[1], *M.M. Sale*[1,2], *I.M. Russell-Eggitt*[3], *D.A. Mackey*[1,4], *M.G. Wirth*[1,4], *L.M. FitzGerald*[1], *M. Shaw*[5], *S. Sharma*[6], *S. Gajovic*[7], *P. Gruss*[7], *S. Ross*[8], *P. Thomas*[8], *A.K. Voss*[9], *T. Thomas*[9], *J. Gécz*[5], *J.E. Craig*[1,4,6]. 1) Menzies Centre for Population Health Research, University of Tasmania, Hobart, Australia; 2) Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, USA; 3) Great Ormond St Hospital for Children, London, UK; 4) Centre for Eye Research Australia, University of Melbourne, Royal Victorian Eye and Ear Hospital, Melbourne, Australia; 5) Department of Cytogenetics and Molecular Genetics, Women's and Children's Hospital, Adelaide, South Australia; 6) Department of Ophthalmology, Flinders University, Flinders Drive, Bedford Park, South Australia; 7) Department of Molecular Cell Biology, Max-Planck-Institute of Biophysical Chemistry, Goettingen, Germany; 8) Murdoch Children's Research Institute, Melbourne, Victoria, Australia; 9) Development and Neurobiology, Walter and Eliza Hall Institute, Parkville, Victoria, Australia.

Nance-Horan Syndrome (NHS) is an X-linked disorder characterized by severe congenital cataracts, dental anomalies, dysmorphic facial features, and in some cases, mental retardation. We have investigated a novel candidate gene within the NHS critical region. The gene was predicted by the GeneScan prediction software and supported by ESTs from relevant tissues in several species. The genomic structure was determined with RT-PCR and 5RACE. Nine exons were detected, spanning 650kb and coding for a 1,630 amino acid protein. Expression was detected in all human and murine tissues tested by RT-PCR and Northern blot. Truncating mutations were identified in 5 pedigrees with NHS, but not in 200 control chromosomes suggesting the gene is causative for NHS. No sequence similarity to known genes or proteins was identified. The developmental expression pattern of this gene of novel class has been further studied using mouse in situ hybridization and a LacZ insertional mutant. These studies strongly suggest key functions in the regulation of eye, tooth, craniofacial and brain development, consistent with the pleiotropic features of NHS.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 36 from 2003 ASHG Annual Meeting

Positional cloning of *COH1*, a novel membrane transport protein mutated in patients with Cohen syndrome. *H.C. Hennies[1], A. Rauch[2], C. Schumi[1], A. Müllner-Eidenböck[3], E. Al-Taji[4], G. Tariverdian[5], K.H. Chrzanowska[6], A. Rajab[7], K.M. Eckl[1], M. Karbasiyan[8], A. Reis[2], D. Horn[8].* 1) Dept Molecular Genetics, Max-Delbrueck Ctr, Berlin, Germany; 2) Inst Human Genetics, Univ Erlangen, Germany; 3) Dept Ophthalmology, Univ Hospital Vienna, Austria; 4) Paediatric Dept, Univ Hospital Vienna, Austria; 5) Inst Human Genetics, Univ Heidelberg, Germany; 6) Dept Medical Genetics, Children's Memorial Health Inst, Warsaw, Poland; 7) Genetic Unit, Ministry of Health, Muscat, Sultanate of Oman; 8) Inst Human Genetics, Charite, Humboldt Univ, Berlin, Germany.

Cohen syndrome is a rare autosomal recessive disorder with a variable clinical picture mainly characterized by mental retardation, microcephaly, typical facial dysmorphism, progressive pigmentary retinopathy, severe myopia, and intermittent neutropenia. The Cohen syndrome locus *COH1* was mapped to chromosome 8q21-q22 before. By homozygosity mapping in families from Germany, Turkey, Poland, and Oman, we placed the gene in a 4.4 cM interval between D8S521 and D8S1749. This 2.1 Mb region contains at least 15 genes. In a transcript spanning more than 150 kb of genomic sequence we identified several mutations in a total of 24 unrelated patients with Cohen syndrome. Patients of a consanguineous Lebanese family showed a homozygous G>T mutation in a splice acceptor site leading to cryptic splicing. Three patients from German and Polish families showed heterozygous frameshift deletions and a nonsense mutation. The impact of various missense mutations has not been assessed so far. At present we have characterized 24 exons of *COH1*. Recent data suggest various splice forms of *COH1* with transcripts of up to 62 exons, and we expect further 5 exons to contain the missing mutations in compound heterozygous patients. Alignment of the deduced peptide sequence showed similarity with VPS13, a protein-sorting protein of yeast involved in intracellular transport. Domain analysis predicted metallopeptidase and inner membrane component motifs. Identification of mutations in *COH1* as the cause of Cohen syndrome will contribute to a better understanding of the disease mechanism and shed light on its intriguing clinical variability.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 37 from 2003 ASHG Annual Meeting

Molecular disease confirmation in a case of Bardet-Biedl syndrome, prenatal diagnosis in a sibling and segregation of genitourinary malformations in unaffected relatives with heterozygous mutation. *B. Hoskins[1], M. Holden[2], S. Taffinder[2], G. Norbury[3], R. Jones[3], W. Van't Hoff[4], P. Pandya[5], R.M. Winter[2], P.J. Scambler[1], P.L. Beales[1].* 1) Molecular Medicine Unit, Institute of Child Health, London, UK; 2) Clinical Genetics Department, Institute of Child Health, London, UK; 3) Clinical Molecular Genetics Laboratory, Great Ormond Street Hospital, London, UK; 4) Department of NephroUrology, Institute of Child Health, London, UK; 5) Fetal Medicine Unit, University College London Hospitals, London, UK.

BBS is characterised by retinal dystrophy, obesity, polydactyly, cognitive impairment, genitourinary malformations and renal dysfunction. Diagnosis is not straightforward, particularly in the young owing to the variable expressivity and progressive nature of diagnostic features. We describe here, molecular confirmation of disease (R275X and Y24X in *BBS2*) in a fifteen month old British boy who did not meet clinical diagnostic criteria. We also report the subsequent prenatal diagnostic testing for his unborn sibling by chorionic villus sampling and molecular analysis. As far as we are aware both these tests represent the first report of molecular and prenatal diagnosis for BBS.

Careful examination of the probands pedigree revealed five relatives with complex genitourinary malformations. Further molecular testing of these individuals confirmed that each harbors a heterozygous Y24X mutation in *BBS2*. None has additional clinical features compatible with a diagnosis of BBS. Three possible mechanisms may account for the co-segregation of GU malformations with the Y24X mutation; 1) this is coincidental 2) that heterozygosity can manifest a partial BBS phenotype mimicking a dominant inheritance pattern or 3) these individuals have additional mutations in other (presumed cis-acting) genes which modify and confine the phenotype to the genito-urinary tract implicating a developmental role for these proteins on Mullerian derivatives. We discuss the significance of these findings in view of the presence of Y24X, a mutation previously implicated in triallelic inheritance.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 38 from 2003 ASHG Annual Meeting

Basal body dysfunction likely underlies the pleiotropic phenotype of Bardet-Biedl syndrome. *N. Katsanis[1,5], S.J. Ansley[1], J.L. Badano[1], O.E. Blacque[2], J. Hill[3], B.E. Hoskins[3], C.C. Leitch[1], J.C. Kim[2], A.J. Ross[3], E.R. Eichers[4], T.M. Teslovich[1], A.K. Mah[2], R.C. Johnsen[2], J.C. Cavender[4], R.A. Lewis[4], M.R. Leroux[2], P.L. Beales[3].* 1) Institute of Genetic Medicine, Johns Hopkins University; 2) Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC; 3) Molecular Medicine Unit, Institute of Child Health, University College London, UK; 4) Departments of Molecular and Human Genetics, Baylor College of Medicine; 5) Wilmer Eye Institute, Johns Hopkins University.

Bardet-Biedl syndrome (BBS) is a genetically heterogeneous, multisystemic disorder characterized primarily by retinal dystrophy, obesity, polydactyly, renal malformations and learning disabilities, and although five BBS genes have been cloned, the molecular basis of this syndrome remains elusive. Here we provide evidence that BBS is likely caused by a defect at the basal body of ciliated cells. We have cloned a novel BBS gene, *BBS8*, which encodes a protein with a prokaryotic domain, *pilF*, involved in pilus formation and twitching mobility. In one family, a homozygous null BBS8 mutation leads to BBS with randomization of left-right axis of body symmetry, a known defect of the nodal cilium. Consistent with a ciliary role for this protein, we have found BBS8 to localize specifically to ciliated structures, such as the connecting cilium of the retina and columnar epithelial cells in the lung; in cells, BBS8 localizes to centrosomes and basal bodies and interacts with PCM1, a protein involved in ciliogenesis. Finally, we demonstrate that all *C. elegans* homologues of the known BBS genes are expressed exclusively in ciliated neurons, and contain regulatory elements for RFX, a transcription factor that modulates the expression of genes associated with ciliogenesis and intraflagellar transport.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 39 from 2003 ASHG Annual Meeting

Characterization of a Mouse Model for Bardet-Biedl Syndrome Type 4. *K. Mykytyn[1,2], R. Mullins[3], D.Y. Nishimura[1], C. Searby[1,2], M. Andrews[1,2], B. Yang[4], E.M. Stone[2,3], V.C. Sheffield[1,2]*. 1) Dept of Pediatrics, University of Iowa, Iowa City, IA; 2) Howard Hughes Medical Institute; 3) Dept of Ophthalmology, University of Iowa, Iowa City, IA; 4) Dept of Obstetrics and Gynecology, University of Iowa, Iowa City, IA.

Bardet-Biedl syndrome (BBS) is a genetically heterogeneous disorder with the primary features of obesity, pigmentary retinopathy, polydactyly, renal and cardiac malformations, mental retardation, and hypogenitalism. Secondary features include diabetes mellitus and hypertension. BBS has been linked to seven loci and the *BBS1*, *BBS2*, *BBS4*, *BBS6* (*MKKS*), and *BBS7* genes have recently been identified. The BBS6 protein sequence shows similarity to chaperonins while the others show no significant similarity to any known proteins. Therefore, it is still not understood how mutations in these genes lead to manifestation of the BBS phenotype. Although BBS is a recessive disorder, it has recently been proposed that some families require three mutant alleles (two at one locus, and a third at a second locus) for manifestation of BBS. In order to elucidate the function of the BBS proteins and investigate complex inheritance of BBS we have begun developing animal models. We have successfully targeted the *Bbs4* gene in mice and are in the process of evaluating the phenotype of mice targeted at one or more of the BBS loci. *Bbs4*$^{-/-}$ mice appear to recapitulate the human phenotype as they become obese, fail to reproduce, and display retinal degeneration. Histological examination of retinas from knockout mice demonstrates that within weeks after birth the photoreceptors begin degenerating in an apoptotic process that leads to the eventual loss of the entire photoreceptor layer. Using the *Bbs4* knockout mouse and other BBS mouse models, we hope to elucidate the function of the BBS proteins and understand the possible genetic interactions. Additionally, this animal model of a Mendelian disorder may prove useful in understanding the processes underlying complex disorders, such as obesity.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 40 from 2003 ASHG Annual Meeting

PLP2, A Novel Candidate Gene for X-Linked Retinitis Pigmentosa. *L. Zhang*[1], *T. Wang*[1], *I.H. Maumenee*[2], *D. Valle*[1,3]. 1) Inst. of Genet. Med; 2) Wilmer Eye Inst., Johns Hopkins Univ; 3) Howard Hughes Med. Inst., Baltimore, MD.

Retinitis pigmentosa (RP) is a group of genetically heterogeneous retinal degenerations with night blindness, progressive reduction of the visual fields and pigmentary retinopathy. X-linked RP (XLRP) is a severe RP subgroup and 2 genes on the X (RP2 and RP3) have been shown to account for ~75% of the patients. We utilized an X-chromosome cDNA microarray to search for additional candidate genes identifying PLP2 as one. We found a missense mutation, PLP2-R6C, in two independent patients with atypical XLRP and not present in 150 control X chromosomes. PLP2 encodes a 17 kD integral membrane protein with four transmembrane domains, that when expressed in cultured cells, localizes to the ER membrane. Using in situ hybridization and immunohistochemical methods, we showed that PLP2 is enriched in the photoreceptor outer segments. By co-immunostaining with known marker proteins and confocal microscopy, we showed that PLP2 is localized specifically on the disc rim of the photoreceptor outer segments. Peripherin-2 and Rom-1 are two well-studied proteins that localize to the disc rim forming homo-and heterotetramers thought to be necessary for the establishment and maintenance of the disc structure. Peripherin-2 mutations cause autosomal dominant RP and together with Rom-1 mutations, digenic RP. Using co-immunoprecipitation in transfected HEK293 cells, we showed that PLP2 interacts with itself and Peripherin-2 but not Rom-1. The PLP2-R6C mutation does not alter these interactions. Our results suggest a working model in which PLP2, like Rom-1, forms heterotetramers with Peripherin-2. We hypothesize that the function of PLP2 and Rom-1 in the retina is redundant, explaining why no phenotype has been associated with isolated Rom-1 mutations. Missense mutations in PLP2 may have a dominant negative effect on the interactions with Peripherin-2, or, like Rom-1, contribute to digenic inheritance. We conclude that PLP2 is likely to play important roles in the pathogenesis of retinal degeneration in a subset of XLRP and possibly, digenic RP patients and is important for the normal structure of the photoreceptor outer segment.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

A New Animal Model of Congenital Stationary Night Blindness (CSNB). *S.H. Tsang[1,2], S. Nuzsinowitz[2], M. Woodruff[2], C.K. Yamashita[2], W.H. Lee[2], G. Fain[2], D.B. Farber[2].* 1) Columbia University, New York, NY; 2) UCLA, Los Angeles, CA.

Many heredity retinal dystrophies manifest as retinitis pigmentosa (RP) or congenital stationary night blindness (CSNB). One of the initial symptoms of RP is night blindness, but the disease later progresses to apoptotic cell death and complete blindness. In contrast, CSNB features only nonprogressing night blindness without loss of cone (day light) vision. These 2 diseases can be caused by different mutations in the same gene, i.e. the subunit of cGMP phosphodiesterase (PDE). Individuals with CSNB in the Rambusch pedigree were found to have the His258Asn (Exon 4) mutation; a similar mutation has not been found in RP patients. Our purpose was to make an animal model carrying His258Asn in PDE to investigate why this mutation does not lead to retinal degeneration.The murine opsin promoter was used to direct expression of wild-type PDE and His258Asn PDE transgenes to the photoreceptors. These constructs were injected into normal oocytes. The resulting founder mice were then crossed with *rd1/rd1* mice that lack PDE to generate progeny that could only synthesize transgenic PDE.While *rd1/rd1* mice have severe photoreceptor degeneration resembling RP, His258Asn mice display features of CSNB, as diagnosed by morphological and physiological studies. The His258Asn mutant construct rescued the rd1/rd1retinal degeneration. However, the His258Asn increased the rate of cGMP hydrolysis, and slightly reduced the light sensitivity of rods. cGMP-PDE activity was 319.2 nmol x min-1 x nmol rhodopsin in the mutant animals compared with 130.1 nmol x min-1 x nmol rhodopsin in wild-type controls. The intensity response function of the rescued rods was shifted rightward by 0.25-0.5 log units.Comparison between *rd1/rd1* and His258Asn PDE mutant mice should allow the dissection of how different abnormalities in photoreceptor signaling lead to divergent phenotypes such as those of RP and CSNB. In His258Asn mice, higher than normal PDE activity may not be sufficient to desensitize the rod photoreceptors. Analyses of possible compensatory mechanisms in response to the His258Asn genetic insult may allow the understanding of the plasticity of the neuronal survival.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Transprenyltranferase (TPT) mutation in patients with coenzyme Q10 biosynthesis deficiency and mitochondrial disorder. *I. Giurgea, P. de Lonlay, D. Cretien, P. Rustin, A. Munnich, A. Rotig.* INSERM U393, Hpital Necker-Enfants Malades, Paris, France.

CoQ10 plays a pivotal role in the mitochondrial respiratory chain as it distributes the electrons between the various dehydrogenases and the cytochrome segments of the respiratory chain. We have identified an inborn error of CoQ10 biosynthesis in two patients born to first cousin parents. Both patients presented with mild intellectual retardation, profound deafness, optic atrophy, valvulopathy and obesity. Enzymological study of the respiratory chain in skin fibroblasts showed that quinone-dependent activities (complex I+III, complex II+III, glycerol-3-P cytochrome c reductase) were in the lowest control values whereas all respiratory chain complexes showed normal activities. CoQ10 deficiency was confirmed by restoration of normal activities upon quinone addition during respiratory chain activities measurement. This deficiency was observed in cultured skin fibroblasts but not in muscle. A genome wide search for homozygosity allowed us to identify several regions on chromosomes 1, 2, 10, 12 and 13, possibly containing the disease-causing gene. One of these regions, between markers D10S1714 and D10S593 (10p12.1), encompassed the gene encoding the transprenyltransferase (TPT), an enzyme involved in assembly of the polyprenoid side chain of CoQ10. Sequencing this gene in all patients allowed to identify a homozygous nucleotide substitution modifying a highly conserved amino acid of the protein (D308E). Both parents were heterozygotes for this mutation and healthy sibs were either heterozygotes or wild-type homozygotes. This mutation was absent from 100 unrelated controls of the same ethnic origin. Functional tests in a Saccharomyces cerevisiae strain deleted for the COQ1 gene, the yeast TPT counterpart, are in process. This work reports the first description of a mutation responsible for CoQ10 deficiency in human.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 43 from 2003 ASHG Annual Meeting

Mutations in the gamma actin gene (ACTG1) cause dominant progressive deafness (DFNA20/26). *K.H. Friderici*[1], *M. Zhu*[1], *T. Yang*[2], *S. Wei*[1], *A.T. DeWan*[3], *I. Belyantseva*[4], *R.J. Morell*[4], *J.L. Elfenbein*[1], *R.A. Fisher*[1], *S.M. Leal*[3], *R.J.H. Smith*[2]. 1) Dept Micro & Molec Gen, Peds & Hum Dev, Audio & Speech Sci, Mich. State Univ. East Lansing, MI; 2) Dept of Otol, Univ. Iowa, Iowa City, IA; 3) Dept Mol & Hum Gen, Baylor Col. Of Med., Houston, TX; 4) NIDCD/NIH, Rockville, MD.

Age-related hearing loss (presbycusis) is a significant problem in the population. Nearly half of those over age 80 have a hearing loss that interferes communication. The genetic contribution to age related hearing loss is estimated as 40-50%. Gene mutations that cause nonsyndromic progressive hearing loss with early onset may provide insight into the etiology of presbycusis. We have identified four families segregating an autosomal dominant, progressive hearing loss phenotype that has been linked to chromosome 17q25.3. The critical interval containing the causative gene was narrowed to approximately 2 million base pairs between markers D17S914 and D17S668. Cochlear expressed genes were sequenced in affected family members. A non-conservative amino acid change in the gamma actin gene (ACTG1) was discovered. Sequence analysis of three additional families that map to the region showed they also carry missense mutations in highly conserved actin domains. These mutations change amino acids that are conserved in all actins from protozoa to mammals and they were not found in more than 100 chromosomes from normal hearing individuals. This is the first description of a mutation in cytoskeletal, or non-muscle, actin. Actin is an exceptionally evolutionarily conserved protein in part because it supports an unusually wide variety of protein-protein interactions. Many of the mutations known to cause either syndromic or non-syndromic deafness occur in genes that interact with actin (eg., the myosins, espin, harmonin). Much of the specialized ultrastructural organization of the cells in the cochlea is based on the actin cytoskeleton. The mutations we have identified occur in various binding domains of actin and are predicted to mildly interfere with bundling, gelation, polymerization or myosin movement and may cause hearing loss by impeding the repair of cochlear cell structures due to damage from noise or aging.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 44 from 2003 ASHG Annual Meeting

Natural selection and molecular evolution in *PTC*, a bitter taste receptor. *S. Wooding*[1], *U.-k. Kim*[2], *J. Larsen*[2], *M.J. Bamshad*[1], *L.B. Jorde*[1], *D. Drayna*[2]. 1) Human Genetics, University of Utah, Salt Lake City, UT; 2) National Institute on Deafness and Communication Disorders, Rockville, MD.

The ability of some individuals to taste phenylthiocarbamide (PTC) is a classic phenotype that has long been known to vary in human populations. This phenotype is of genetic, epidemiologic and evolutionary interest because the ability to taste PTC is correlated with the ability to taste other bitter substances, many of which are toxic. Thus, variation in PTC perception may reflect variation in dietary preferences throughout human history, and could correlate with susceptibility to diet-related diseases in modern populations. The recent localization of the *PTC* gene, which accounts for up to 85% of phenotypic variance in PTC perception, raises basic questions about the evolution of this gene. To address these questions, we examined DNA sequence variation at the 1kb *PTC* locus in 258 chromosomes collected from African, Asian, European, and North American populations. We analyzed these data using new statistical tests for natural selection that take into account the potentially confounding factor of human population history. Two haplotype clusters corresponding to "taster" and "non-taster" haplotypes were found. These clusters had similar frequencies across Africa, Asia, and Europe. Genetic differentiation between these continents was unusually low and did not differ significantly from zero (F_{ST}=0.003, $p > 0.40$). Tajima's T and Fu and Li's D and F statistics were significantly greater than expected due to an excess of intermediate-frequency variants (T=1.39, D=-1.40, F=-0.53, $p < 0.01$), but only when recent population growth was taken into account. These findings are consistent with the hypothesis that the taster and non-taster allele clusters have been maintained for an exended period by balancing natural selection. The prevalence of the non-taster haplotype in populations worldwide suggests the hypothesis that this haplotype encodes a functional receptor for a toxic bitter substance other than PTC. (Support: NIH ES12125 to SW, NIH GM59290 to LBJ, NIH Z01-000046-04 to DD).

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Null Leukemia Inhibitory Factor Receptor (LIFR) mutations in Stüve-Wiedemann/Schwartz-Jampel type 2 syndrome. *N. Dagoneau[1], D. Scheffer[1], C. Huber[1], L.I. Al-Gazali[2], M. Di Rocco[3], A. Godard[4], J. Martinovic[1], A. Raas-Rothschild[5], S. Sigaudy[6], S. Unger[7], A. Superti-Furga[8], M. Le Merrer[1], J. Bonaventure[1], A. Munnich[1], L. Legeai-Mallet[1], V. Cormier-Daire[1].* 1) Department of Medical Genetics and INSERM U393, Hopital Necker, Paris, France; 2) Department of Pediatrics, UAE University, Al Ain, United Arab Emirates; 3) Second Unit of Pediatrics, Istituto G.Gaslini, Genoa, Italy; 4) INSERM U463, Nantes, France; 5) Department of Genetics, Hadassah University Medical Center, Jerusalem, Israel; 6) Hopital d'Enfants de la Timone, Marseille, France; 7) Division of Clinical and Genetic Metabolics, University of Toronto, Toronto, Canada; 8) Department of Pediatrics, University of Lausanne, Lausanne, Switzerland.

Stüve-Wiedemann syndrome (SWS) is a severe autosomal recessive condition characterized by bowing of the long bones, with cortical thickening, flared metaphyses with coarsened trabecular pattern, camptodactyly, respiratory distress and hyperthermic episodes responsible for early lethality. Clinical overlap with Schwartz-Jampel type 2 syndrome (SJS2) has suggested that SWS and SJS2 could be allelic disorders. Studying a series of 18 SWS/ SJS2 families, we have mapped the disease gene to chromosome 5p13.1 at locus D5S418 (Zmax= 10.66 at theta=0) in a genetic interval of 3.2Mb. This region encompasses the Leukemia Inhibitory Factor Receptor (LIFR) which was considered as a good candidate gene based on the LIFR-/-mice which present with reduction of fetal bone volume, increased number of osteoclasts and perinatal death. The direct sequencing of LIFR allows us to identify thirteen distinct mutations with 12/13 mutations leading to a premature stop codon. Our study shows first that SWS and SJS2 represent a single genetic entity. Moreover, functional studies indicate that these mutations alter the stability of LIFR mRNA transcripts resulting in the absence of the LIFR protein and in the impairment of the JAK/STAT3 signalling pathway in patient cells.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 46 from 2003 ASHG Annual Meeting

A new syndrome caused by a novel loss-of-function mutation in *FGFR3*. *R. Toydemir[1], N. Longo[2], A. Brassington[1], P. Bayrak-Toydemir[3], P. Krakowiak[4], L.B. Jorde[1], M. Bamshad[1,2]*. 1) Dept of Human Genetics, Univ Utah, SLC, UT; 2) Dept of Pediatrics, Univ Utah, SLC, UT; 3) DNA Diagnostic Lab, Univ Utah, SLC, UT; 4) Dept of Pediatrics, Univ Arkansas, Little Rock, AK.

We report a kindred in which 20 individuals in 7 generations were affected with bilateral sensorineural hearing loss, camptodactyly, joint hyperextensibility, tall stature, thoracolumbar kyphoscoliosis, microcephaly and developmental delay. Radiographic findings included tall vertebral bodies, broad femoral metaphyses, and osteochondromas of the femur, tibia, and phalanges. Middle and inner ear structures were normal via CT exam. Pedigree analysis suggested that this disorder is transmitted in an autosomal dominant pattern. A genome-wide scan yielded a maximum LOD score of 3.76 with a marker on chromosome 4p. Haplotype analysis defined a critical interval between D4S3038 and D4S431 that contained several candidate genes including fibroblast growth factor receptor 3 (*FGFR3*). Mice homozygous for a null mutation of *FGFR3* develop elongated femurs and vertebral bodies, hip dysplasia, and hearing loss. Because of these overlapping phenotypic similarities, we screened *FGFR3* for mutations by direct DNA sequencing. A GA missense mutation at nucleotide position 1862 that causes a substitution of histidine for arginine at amino acid position 621, a highly conserved residue in the catalytic loop, was found in all affected family members. This mutation was not found in unaffected family members or 150 control chromosomes from an ethnically matched population. R621H is predicted to cause a loss-of-function by interfering with the transfer of phosphate to its target sites. This loss-of-function is unlikely to result from haploinsufficiency because hemizygous deletion of *FGFR3*, which frequently occurs in Wolf-Hirschhorn syndrome, is not associated with similar skeletal defects. In addition, analysis of primary cells and CHO cells transfected with the mutant receptor reveals normal expression patterns and cellular localization. We hypothesize that R621H causes loss of function by a dominant negative mechanism in which hetero- and homodimers incorporating mutant forms of *FGFR3* have diminished kinase activity.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 47 from 2003 ASHG Annual Meeting

Homozygote Matrilin 3 mutations in a novel form of Spondylo-Epi-Metaphyseal dysplasia. *Z. Borochowitz[1], D. Scheffer[2], V. Adir[1], N. Dagoneau[2], A. Munnich[2], V. Cormier-Daire[2].* 1) The Simon Winter Institute for Human Genetics, Bnei-Zion Medical Center, Haifa, Israel; 2) Department of Medical Genetics and INSERM U393, Hopital Necker, Paris, France.

We report a novel form of autosomal recessive Spondylo-Epi-Metaphyseal dysplasia (SEMD) in a large consanguineous family of Arabic-Muslim origin. All five affected individuals presented with disproportionate early-onset dwarfism, bowing of the lower limbs, lumbar lordosis and normal hands. Skeletal findings included short wide and stocky long bones with severe epiphyseal and metaphyseal changes, hypoplastic iliac bones and flat-ovoid vertebral bodies. Genome wide homozygosity mapping mapped the disease locus gene to chromosome 2p25-24 with a maximum lod score of 3.46 at locus D2S305. Two recombination events defined the minimal critical region interval between loci D2S320 and D2S215 (2.24 Mb). Using a candidate gene approach, we identified, in all affected individuals, an homozygous substitution (973TA) in the gene encoding for Matrilin 3 (MATN3), a cartilage specific protein. The mutations changed a cysteine into a serine in the EGF-domain of MATN3, in a region highly conserved across species. Interestingly, heterozygous missense MATN3 mutations have been previously reported in two families with multiple epiphyseal dysplasia (MED). In the family reported here, none of the heterozygous parents complained of knee pain and radiographic evaluation did not reveal any degree of epiphyseal dysplasia. Although mutations in MATN3 causing either MED or this novel form of SEMD are all missense, their consequences are certainly distinct as they are clearly not located in the same domains (ie von Willebrand/EGF domains). We conclude that homozygous MATN3 mutations are responsible for this novel form of SEMD.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Mutations in the p97 gene cause familial inclusion body myopathy associated with Paget disease of the bone and frontotemporal dementia. *G.D.J. Watts*[1], *J. Wymer*[1], *S. Mehta*[1], *S. Mumm*[2], *M. Whyte*[2], *A. Pestronk*[3], *D. Darvish*[4], *V.E. kimonis*[1]. 1) Division of Genetics, Children's Hospital Boston, Harvard Medical School, Boston, MA; 2) Division of Bone and Mineral Diseases, Washington University School of Medicine and Barnes-Jewish Hospital Research Institute, St. Louis MO; 3) Department of Neurology, Washington Univ. School of Med., St Louis, MO; 4) HIBM Research Group, 16661 Ventura Blvd., #311, Encino, CA.

We report, in 9 families, molecular findings for a new autosomal dominant disorder associated with inclusion body myopathy clinically resembling limb girdle muscular dystrophy, Paget disease of bone in the majority and frontotemporal dementia in a third of individuals. The critical locus for this unique disorder termed IBMPFD (MIM 605382) on 9p21.1-p12, spans 5.5Mb. This is a gene rich locus and encompasses two other myopathies; autosomal recessive inclusion body myopathy (IBM2) and a rare nemaline myopathy. We have identified five missense mutations within the p97 gene in 9 families with IBMPFD. p97, which is also referred to as CDC48 and VCP (Valosin Containing Protein), is widely expressed and contains two AAA ATPase domains. The p97 protein has been implicated in two distinct and crucial cell pathways, namely membrane biogenesis and targeted protein degradation. The mutations associated with IBMPFD were found to cluster in the N-domain and potentially define a new domain having a critical role in skeletal muscle, osteoclasts, frontal and anterior temporal lobe function.

With growing evidence for disruption of the ubiquitin pathway being involved in the pathological effects for muscle (limb-girdle muscular dystrophy 2H: TRIM32), Paget (Sequestosome 1) and neurodegenerative disease such as CMT1C, mutations in VCP may account for a significant proportion of patients with isolated myopathy, Paget disease of bone and frontotemporal dementia and may define a new ubiquitin-based mechanism of regeneration/stress response leading to inclusion body formation.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 49 from 2003 ASHG Annual Meeting

Spinal deformity in *Crtap-/-* mice. *R. Morello[1], T. Bertin[1], Y. Chen[1], P. Castagnola[3], R. Cancedda[3], B. Lee[1,2]*. 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX; 3) National Institute for Cancer Research, Genoa, Italy.

Recently we described the isolation and characterization of a novel protein, Crtap, highly expressed in *in vitro* chicken hypertrophic chondrocytes as compared to proliferating chondrocytes in vitro. Crtap is ubiquitously expressed at low levels but at high levels in cartilage during the chick embryonic development. It has homology to some signaling proteoglycans. We isolated the mouse orthologous gene of *Crtap* and confirmed its expression in chondrocytes at E15.5 by in situ hybridization. To understand its function we inactivated the gene in mouse ES cells and generated chimeric mice. Heterozygous *Crtap+/-* mice are asymptomatic. Northern analysis of *Crtap-/-* mice confirmed the absence of Crtap mRNA from total embryo RNA. Null mice are born viable at the expected mendelian ratio and show no obvious abnormalities at birth. However, by 3-4 months of age they begin to develop a moderate kyphosis that becomes pronounced at 6 months of age. Faxitron analyses confirmed the deformity of the spine at the thoracic level, in the absence of other obvious patterning defects. Histological analysis revealed a disorganized growth plate with abnormal chondrocyte morphology in both vertebral bodies and long bones. Moreover, there is significant osteoporosis. These data point to a critical role for *CRTAP* for maintaining skeletal homeostasis and playing a possible role in the pathogenesis of human spine deformities.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 50 from 2003 ASHG Annual Meeting

Regulation of bone formation by Wnt signaling. *M.S. Patel[1], D.A. Glass II[1], F. Long[2], M.M. Taketo[3], A.P. McMahon[4], G. Karsenty[1]*. 1) Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Bone Mineral Diseases, Washington University, St. Louis, MO; 3) Department of Pharmacology, Kyoto University, Kyoto, Japan; 4) Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA.

Lrp5 loss-of-function mutations lead to low bone mass with fractures while Lrp5 gain-of-function mutations lead to high bone mass and fracture resistance thus identifying Lrp5 as one of the most important regulators of skeletal physiology discovered to date. Lrp5 is thought to signal in response to Wnt proteins but this has not been demonstrated in vivo. To better understand the nature of the Lrp5 signaling pathway we directly activated the canonical Wnt signaling pathway only in osteoblasts in vivo. These mice, harboring a constitutively active allele of -catenin, have a high bone mass phenotype that, when crossed onto an Lrp5 null background, completely rescues the low bone mass of Lrp5 deficiency. In a converse experiment, we generated an osteoblast-specific deletion of -catenin; our preliminary analyses indicate that these mice recapitulate the low bone mass of Lrp5 deficiency. To further demonstrate the role of -catenin in Lrp5 signaling, we also generated transgenic mice expressing the high bone mass $LRP5^{G171V}$ allele in osteoblasts. If -catenin is indeed downstream of Lrp5, then selective deletion of -catenin in the osteoblasts of these high bone mass $LRP5^{G171V}$ mice should rescue their phenotype. To test this we are crossing $LRP5^{G171V}$ transgenics to mice with a conditionally null allele of -catenin in the presence of an (I)1 collagen-cre transgene. Lastly, we used a candidate gene approach to identify the transcriptional partner for -catenin in osteoblasts. These gene deletion experiments identified a member of the Lef/Tcf family as the putative DNA binding partner of -catenin. Our results so far strongly suggest that Lrp5 signals through the canonical Wnt signaling pathway in vivo, thus identifying Wnt proteins as critical regulators of differentiated osteoblast function.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 51 from 2003 ASHG Annual Meeting

***Dll3-Notch1* double mutant mice are a model for congenital scoliosis and craniofacial disorders.** *K. Kusumi*[1,2,3,4], *S.A. Stevens*[3], *M.S. Mimoto*[3], *S.L. Dunwoodie*[6], *K.M. Loomes*[1,3,5]. 1) Dept Ped, Univ of Penn School of Med, Philadelphia, PA; 2) Dept Cell & Dev Biol, Univ of Penn School of Med, Philadelphia, PA; 3) Div Hum Genet & Mol Biol, Children's Hosp of Philadelphia, PA; 4) Div Orthop Surg, Children's Hosp of Philadelphia, PA; 5) Div Gastroenterol & Nutr, Children's Hosp of Philadelphia, PA; 6) Victor Chang Cardiac Res Inst, Sydney, Australia.

Congenital scoliosis is a lateral curvature of the spine, caused by single or multiple defects during vertebral formation. The genetic etiology of congenital scoliosis is largely unknown, but mutations in the notch ligand *DLL3* have recently been identified in spondylocostal dysostosis, a severe vertebral disorder. Genes in the notch signaling pathway regulate the formation of somites, the embryonic precursors to vertebrae and ribs. To date, while targeted mouse mutant models (*Notch1, Dll1, Dll3, Psen1, Hes7* and *Lfng*) exist for severe, global vertebral defects, there has been a lack of a model with limited, localized defects similar to congenital scoliosis.

We have generated $Dll3^{neo}/Notch1^{tm1Con}/+$ double heterozygous animals. At birth, double mutants display vertebral and rib defects similar to congenital scoliosis, with full penetrance (n15). Double heterozygous mutants also display maxillary and mandibular anomalies, producing a micrognathic phenotype. We have also observed a high rate of edema, leading to 50% increase in birth weight. Histological and gross pathological analyses are currently being conducted. At 16.5 days *post coitum* (dpc), we observe deformed double heterozygous embryos, and at 10.5 dpc, we are characterizing markers for somite patterning. *Notch1* or *Dll3* single heterozygous animals do not display any of these phenotypes. Generation of *Notch1-Dll3* double mutant mice with congenital scoliosis-type defects provides a unique model for developmental analysis of this birth defect. These findings also point to a potential role for *DLL3-NOTCH1* interactions in congenital scoliosis patients.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 52 from 2003 ASHG Annual Meeting

The BMP2 Gene Contributes to Bone Density and Osteoporotic Fractures. *U. Styrkarsdottir[1], J-B. Cazier[1], O. Rolfsson[1], H. Larsen[1], E. Bjarnadottir[1], V.D. Johannsdottir[1], M.S. Sigurdardottir[1], Y. Bagger[2], C. Christiansen[2], K. Jonasson[1], M.L. Frigge[1], A. Kong[1], J.R. Gulcher[1], G. Sigurdson[3], K. Stefansson[1].* 1) deCODE genetics, Reykjavik, Iceland; 2) Center for Clinical and Basic Research A/S, Ballerup, Denmark; 3) National University Hospital, Reykjavik, Iceland.

We have mapped to chromosome 20 a gene contributing to osteoporosis (OP) and isolated it using a case-control association analysis. Linkage analysis in a large number of extended Icelandic OP families showed statistically significant linkage to chromosome 20p12.3 (multipoint allele sharing LOD 4.96, P-value 8.8 x 10-7). The 9 cM region defined within the drop of one in LOD score corresponds to a 2.2 MB segment containing 6 known genes, the BMP2 gene being a strong candidate. However, expression analysis showed that four of the genes in the region, including BMP2, are expressed in bone marrow or in an osteoblast cell line, and can therefore all be considered strong candidates. To decide which gene contributed most to OP we determined the segment that showed the greatest association in a case-control association study. We included 705 patients and 700 unrelated controls in the initial analysis. A very dense set of polymorphic markers distributed across the one-LOD drop, including both microsatellites and SNPs, was used. Single-marker association and haplotype analysis showed the strongest association to the region of the BMP2 gene. We found a missense variant in the BMP2 gene to be significantly associated with OP. We also found two independent SNP haplotypes, defined by 2 and 3 SNPs, respectively, covering the BMP2 gene and showing association with OP. Thus we have found three independent variants in the BMP2 gene to be associated with OP in Iceland, with risk ratios ranging from 2 to 6. These variants are carried by over 30% of OP fracture patients and show association to many definitions of OP including pre- and postmenopausal low BMD. Furthermore, we confirmed the BMP2 association in a Danish cohort of postmenopausal women with comparable risk ratios. Thus, BMP2 gene variations appears to be a major risk factor for OP and OP fractures and represents the first osteoporosis gene discovered using positional cloning.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 53 from 2003 ASHG Annual Meeting

Elevation of CUGBP1 disorders myogenesis in Myotonic Dystrophy 1. *L. Timchenko*[1], *R. Patel*[1], *P. Iakova*[2], *L. Qian*[1], *Z-J. Cai*[1], *N. Timchenko*[2]. 1) Dept Cardiovascular Sci, Baylor Col Medicine, Houston, TX; 2) Dept Pathology and Huffington Center on Aging, Baylor Col Medicine, Houston, TX.

Myotonic Dystrophy 1 (DM1) is the most common form of neuromuscular disease caused by an expansion of untranslated CTG repeats in the 3 UTR of DMPK gene on chromosome 19q. Molecular studies showed a complex pathogenesis for DM1. Cardiac defects and cataracts in DM1 are caused by CTG repeats, while myotonia, a delay of myogenesis and unusual resistance to insulin are mediated by RNA CUG repeats of the mutant DMPK mRNA via specific CUG RNA-binding proteins. Two families of CUG-binding proteins were identified: CUGBP and MBNL. Misregulation of CUGBP1 is associated with three symptoms of DM1: myotonia (Charlet-B et al, Mol Cell, 2002), the delay of skeletal muscle differentiation (Timchenko et al, 2001, Mol Cell Biol) and the insulin resistance (Savkur et al, 2001, Nat Genet). In DM1, CUGBP1-DMPK mRNA complexes protect CUGBP1 from degradation leading to the elevation of CUGBP1 protein levels. To test the role of CUGBP1 elevation in DM1 pathology, we generated transgenic mice that overexpress CUGBP1 mainly in skeletal muscle. Analyses of CUGBP1 transgenic mice revealed that genetically un-programmed elevation of CUGBP1 disrupts myogenesis. The severity of muscle disruptions is proportional to the levels of CUGBP1 elevation. Mutant mice with the levels of CUGBP1 comparable to those in patients with congenital DM1 have significant delay in muscle development. CUGBP1 transgenic mice with the levels of CUGBP1 similar to that in adult DM1 patients develop muscular dystrophy. CUGBP1 increases translation of key regulators of muscle differentiation, myocyte enhancer factor 2A, MEF2A, and p21 by direct interactions with corresponding mRNAs. Examination of these targets in skeletal muscle of CUGBP1 transgenic mice and in DM1 patients revealed a significant induction of MEF2A and p21. These data are consistent with the hypothesis that the un-programmed elevation of CUGBP1 in DM1 muscle increases p21 and MEF2A levels at wrong time of myogenesis disordering muscle development and function.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 54 from 2003 ASHG Annual Meeting

Replication Inhibitors Specifically Modulate Instability of an Expanded CTG Repeat Tract at the Myotonic Dystrophy Locus in Patient Cells. *C.E. Pearson[1,2], Z. Yang[1], R. Lau[1], D. Chitayat[3], J.L. Marcadier[1,2].* 1) Dept Genetics & Genomic Biol, Hosp Sick Children, Toronto, ON, Canada; 2) University of Toronto; 3) University Health Network.

Gene-specific (CTG)(CAG) repeat expansion is associated with at least 14 human neurodegenerative and neuromuscular diseases, including Huntingtons disease and myotonic dystrophy (DM1). Most of our understanding of trinucleotide instability is from non-human models which have presented mixed results, supporting replication errors or processes independent of cell division. Nevertheless, the mechanism occurring at any of the disease loci in patient cells is poorly understood.

Using DM1 patient-derived fibroblasts, we have shown that spontaneous expansion of the diseased (CTG)216 allele occurred in proliferating but not quiescent cells. Furthermore, cells were treated with agents known to alter DNA synthesis but not to directly damage DNA. Inhibiting replication initiation with mimosine had no effect upon repeat instability. Inhibiting both leading and lagging strand synthesis with aphidicolin, or blocking only lagging strand synthesis with emetine significantly enhanced CTG repeat expansions. Strikingly, only the expanded DM1 allele was altered, leaving the normal allele, (CTG)12 and other repeat loci unaffected. Standard and the highly sensitive small-pool PCR revealed that inhibitors significantly enhanced 3-fold the magnitude of short expansions in most cells, while 11-25% of cells experienced gains of 122-170 CTG repeats. Our results support a role for the perturbation of chromosomal replication fork dynamics in DM1 CTG expansions within patient fibroblasts.

This is the first report that repeat length alterations specific to a mutated disease allele can be modulated by exogenously added compounds. The identification of compounds that specifically alter repeat instability at a disease locus provides insight for research aimed at developing pharmacological agents that would intervene with the mutation process to slow pathogenesis in man.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 55 from 2003 ASHG Annual Meeting

Unique phenotype associated with biallelic *BRCA2* mutations includes high risk of brain tumors, Wilms tumors and early onset acute leukemia in children with Fanconi anemia. *A.D Auerbach[1], O. Levran[1], B. Mullaney[2], D.I. Kutler[3], K. Mah[3], A. Deffenbaugh[2], T. Scholl[2], M. Berwick[3], M.L. MacMillan[4], K. Offit[3], J.E. Wagner[4], H. Hanenberg[5].* 1) Lab Human Gen & Hematology, Rockefeller Univ, New York, NY; 2) Myriad Genetic Labs, Salt Lake City, UT,; 3) Memorial Sloan-Kettering Cancer Center, New York, NY; 4) Univ of Minnesota, Minneapolis, MN, USA; 5) Heinrich Heine Univ, Dsseldorf, Germany.

It has recently been reported that the gene associated with the FA-D1 Fanconi anemia (FA) complementation group is *BRCA2*. Here we expand on the clinical relevance of this discovery. Direct DNA sequencing of the coding regions and intron/exon junctions of *BRCA2* was performed on genomic DNA from 28 International FA Registry (IFAR) kindreds. No pathogenic *BRCA2* mutations were found in 17 kindreds; FA complementation groups A, C, D2, E, F or G were excluded in 16 of these, based on studies using cDNA-containing retroviral vectors. At least one pathogenic *BRCA2* mutation was found in 13 patients from 11 kindreds as follows: (1) 6174delT/C3069X; (2) 6174delT/C3069X; (3) E1308X/Q3066X; (4) 5301insA/I2490T; (5) 886delGT/6174delT; (6) 886delGT/L2740X; (7) 4093del4/G2738D; (8) IVS7+2T>G/IVS7;2T>G; (9) IVS7+2T>G/5164del4; (10) IVS7+1G>A/Y1894X; (11) IVS7+1G>A/Y1894X; (12) 2041insA/W2626C; (13) 2816insA/unknown. Median age of diagnosis of brain tumors (primarily medulloblastomas) was 3.5 years (patients 1-5). Patients 6 and 7 had Wilms tumors. Onset of acute leukemia (patients 8-13) was by the age of 5 years in all cases. The median time to development of leukemia among the *BRCA2* patients is 2.2 years (mean=2.5 years) compared to 13.4 years (mean=14.6 years) for the rest of the patients reported to the IFAR with AML (P0.0001). The finding of *BRCA2* mutations in children with FA and brain tumors, Wilms tumors and early onset leukemia is distinctive and constitutes a new syndromic association. For individuals carrying a germline *BRCA2* mutation who are planning children with a partner of Ashkenazi Jewish descent, the high carrier frequency of BRCA2*6174delT in this population and the potential 25% risk for an offspring with FA, are sufficient to justify genetic counseling.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 56 from 2003 ASHG Annual Meeting

Cytogenetic characteristics of malignancies in Fanconi D1-BRCA2 patients. *B. Hirsch*[1], *M. Dolan*[1], *Y. Messinger*[2], *B. Bostrom*[2], *S. Sencer*[2]. 1) Lab Medicine & Pathology, Univ Minnesota Medical Sch, Minneapolis, MN; 2) Pediatric Hematology-Oncology, Children's Hospitals and Clinics, Minneapolis and St Paul, MN.

Fanconi anemia (FA) is an autosomal recessive disease classically associated with variable congenital anomalies, bone marrow failure and predisposition to malignancy. Eight different complementation groups (A, B, C, D1, D2, E, F, and G) have been identified and seven FA genes have been cloned. The FA-D1 subgroup has been shown to be due to biallelic mutation of the BRCA2 gene. Our studies of two FA-D1 kindreds suggested that FA-D1 patients may present with malignancies at earlier ages of onset than typical for FA. Further, the presenting malignancies were solid tumors (Wilms, medulloblastoma) and T-lineage acute lymphoblastic leukemia (T-ALL), malignancies that are relatively rare among FA patients reported in the literature. The cytogenetics of these patients' malignant processes were complex; each involved two or more abnormal clones, and each clone had multiple abnormalities. The T-ALL was particularly striking, with a highly complex karyotype including deletions, duplications, and balanced and unbalanced translocations, none of which were common recurring abnormalities in T-ALL of childhood. The degree of instability in this de-novo T-ALL was similar to that seen in the therapy-associated acute myeloid leukemia (t-AML) that developed in one of these FA-D1 patients within one year after therapy for a solid tumor. In this t-AML, while certain of the aberrations (5q loss, 17p loss) are known recurring abnormalities, more numerous novel aberrations were also identified. The breakage and rearrangement seen in these tumors may represent a counterpart to the unusually high spontaneous chromosomal instability that we observed in blood and/or fibroblasts from these patients. The etiology of many early childhood tumors and T-ALL is unknown. In combination with clinical assessment, the findings of early childhood solid tumors or T-ALL characterized by early age of onset, complex and unusual karyotypes may warrant consideration of evaluation of FA.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 57 from 2003 ASHG Annual Meeting

siRNA Depletion of BRCA1 or BRCA2 Produces Genome Instability with Interstand Crosslinks. *R. Moses, D. Bruun, A. Folias, Y. Akkari, Y. Cox, S. Olson.* Oregon Health & Science University Portland, ORe.

BRCA1 and BRCA2 tumor suppressor proteins are understood to act in repair of double strand breaks (DSBs) and maintenance of genome stability. We have used siRNA to transiently deplete these proteins in order to test whether they function in interstrand crosslink (ICL) repair. The use of siRNA permits depletion in any cell line, and avoids problems of cell lethality, allowing evaluation of single and doubly defective cells for ICL repair. Therefore, it is possible to ask if ICL repair is altered in Fanconi anemia (FA) cells with loss of BRCA1 or BRCA2. BRCA1 co-localizes with the FA complex, and is required for FANCD2 mono-ubiquitination, so depletion of BRCA1 would be expected to prevent FA pathway function. However, BRCA1 also might act in additional ICL repair responses. BRCA2 is the FANCD1 protein, so it would be expected to act in the FA pathway. It also might act in additional ICL repair pathways, since it is associated with the RAD51 protein and acts in DSB repair. We find that depletion of either BRCA1 or BRCA2 produces a type of chromosomal instability in normal human fibroblasts indistinguishable from FA cells after ICL formation. However, the extent of chromosomal breakage or quadriradial formation was always greater for a given dose of crosslinker in cells depleted for BRCA1. When FA fibroblasts were depleted for BRCA1 or BRCA2, the genome instability was increased with BRCA1 depletion, but not BRCA2 depletion. Thus, BRCA1 functions in more than just the FA pathway for genome maintenance following ICL damage, but BRCA2 appears to act only in the FA pathway. Depletion of BRCA1 causes a marked decrease of ubiquitination of FANCD2. However, BRCA2 is not needed for normal ubiquitination of FANCD2 after DNA damage. Thus, BRCA2 is epistatic to FA genes for ICL repair, but not for damage-induced modification of FANCD2 and thus may act downstream from FANCD2.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

A 100 kb block encompassing BRCA1. *J.R. Kidd, W.C. Speed, A.J. Pakstis, K.K. Kidd.* Department of Genetics, Yale University School of Medicine, New Haven, CT.

The early onset breast cancer 1 gene (BRCA1) spans about 84kb at 17q21. Preliminary results from our study of normal variation for 38 populations (averaging 53 individuals/population) at BRCA1 cover 237kb, including BRCA1, with 13 non-coding SNPs. The SNPs average 12kb apart across the gene and about 25kb apart in flanking regions. Heterozygosities across the gene are generally high (30-50%) in most populations studied especially for those SNPs within the gene, but 4 of 6 flanking SNPs vary usefully only in African populations and are mostly fixed elsewhere. A 100 kb segment of 8 markers encompassing the entire gene and extending ~20 kb downstream behaves in most of the world as a block with virtually no evidence of recombination. Two haplotype lineages diverge from the now-absent ancestral sequence. (No definite evidence has been found in the 38 populations for the primate ancestral haplotype which was determined by typing/sequencing samples of several other ape species.) Outside of Africa the two most derived haplotypes predominate but in Africa several of the intermediate forms accumulating mutations still exist. A third haplotype has common frequencies (9 to 27%) in Native American populations but was not found to be present elsewhere. This Native American haplotype can be derived by recombination from the two haplotypes most common worldwide and may well represent a single event coinciding historically with the initial wave of immigrants to the Americas. The evidence so far places the crossover in a 7.3 kb interval extending from introns 15 to 18. Even with evidence of some recombination at the ends of the block, one 13-site haplotype (237kb) is homozygous in over 30% of ~1600 non-African individuals, a remarkable level of global uniformity. In general, ~50% of non-Africans had 13-marker phenotypes indicating unambiguously the haplotypes present. Because several of the intermediate forms are still common in Africa, various LD statistics show different pairwise patterns. Supported in part by NIH GM57672.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Germline *PTEN* promoter mutations and deletions in Cowden/Bannayan-Riley-Ruvalcaba syndrome result in aberrant PTEN protein and dysregulation of the PI3-kinase/Akt pathway. *X.P. Zhou, K.A. Waite, R. Pilarski, M.J. Fernandez, H. Hampel, C. Eng*. Human Cancer Genetics, Ohio State Univ, Columbus, OH.

Germline intragenic mutations in *PTEN* are associated with 80% of Cowden syndrome (CS) patients, and 60% of Bannayan-Riley-Ruvalcaba syndrome (BRRS) patients. The underlying genetic causes remain to be determined in a considerable proportion of classic CS and BRRS without a PCR-detectable *PTEN* mutation. We hypothesized that gross gene deletions and mutations in the *PTEN* promoter might alternatively account for a subset of apparently mutation-negative CS and BRRS patients. Using real time quantitative multiplex PCR techniques, we identified 3 germline hemizygous *PTEN* deletions in 122 apparently intragenic mutation-negative classic CS (N=95) or BRRS (N=27). Fine mapping using markers within and flanking *PTEN* suggested that one deletion encompassed the whole gene and the other 2 included exon 1 and encompassed exons 1 to 5, respectively. Two deletion cases carried the diagnosis of BRRS and one BRRS/CS overlap (features of both). Thus 3/27 (11%) BRRS or BRRS/CS-overlap patients had *PTEN* deletions. Analysis of the *PTEN* promoter revealed 9 cases (7.4%) harboring heterozygous germline mutations. All 9 had classic CS, representing almost 10% of all CS subjects. Eight had breast cancers and/or benign breast tumors, but otherwise mild features. PTEN protein analysis, from one deletion positive and five *PTEN* promoter mutation positive samples, revealed a 50% reduction in protein and multiple bands of immunoreactive protein, respectively. In contrast, control samples showed only the expected band. Further, an elevated level of phosphorylated Akt was detected in the five promoter mutation positive samples, compared to controls, indicating an absence of or marked reduction in functional PTEN. These data suggest that BRRS and CS patients without PCR-detected intragenic *PTEN* mutations be offered clinical deletion analysis and promoter mutation analysis, respectively.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 60 from 2003 ASHG Annual Meeting

No PTEN protein and altered downstream signaling due to heterozygous germline *PTEN* mutations in Cowden syndrome. *K.A. Waite[1], R. Pilarski[1], Y. Luo[2], X-P. Zhou[1], S. Lemeshow[2], C. Eng[1].* 1) Clinical and Human Cancer Genetics Programs and; 2) Center for Biostatistics, The Ohio State University, Columbus, Ohio.

PTEN, a tumor suppressor on 10q23.3, has been implicated in a variety of human cancers including Cowden syndrome (CS) and Bannayan-Riley-Ruvalcaba syndrome (BRRS). The protein product PTEN is a dual specificity phosphatase, dephosphorylating both lipid and protein substrates and has been shown to regulate the phosphatidylinositol-3 kinase (PI3K)/Akt pathway. Proper PTEN signaling leads to G1 cell cycle arrest and apoptosis. Germline mutations in *PTEN* have been well documented, however, the biochemical consequences of such mutations, in patient studies, has not been systematically investigated. We therefore set out to investigate the biochemical and molecular bases of CS and BRRS in 49 patients. We found that 24 (96%) of *PTEN* mutation positive patients had at least 50% PTEN protein levels. Further, of the 24, 17 also had no expression of the wild-type allele (complete PTEN protein null) in the setting of normal RNA levels. Since active PTEN results in the decrease of phosphorylated Akt (P-Akt), we analyzed P-Akt levels and found that 68% of *PTEN* mutation positive and 42% of PTEN mutation negative samples had elevated levels of P-Akt. SAM68 is a protein shown to regulate the PI3K pathway. Because PTEN also regulates this pathway, we analyzed SAM68 in CS/BRRS patient samples. We found that mutation positive patients had an increase in SAM68 levels. Statistical analysis demonstrated that there is a correlation between the levels of PTEN and the occurrence of breast and thyroid cancer and the development of trichilemmomas. In addition, there was a correlation between the levels of SAM68 and the occurrence of breast cancer. In conclusion, we found that germline mutations in *PTEN* adversely affect the expression of PTEN protein. Additionally, we found that western analysis of PTEN protein to be useful for initial determination of mutation status, perhaps more sensitive than mutation analysis, as well as an indicator of component cancer development. Taken together, we propose that analysis of PTEN and SAM68 protein levels could provide beneficial information for patient care.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 61 from 2003 ASHG Annual Meeting

Evaluation of Breast Cancer Risk Assessment Packages in the Family History Evaluation and Screening Programme. *D.G Evans[1], E. Amir[1], A. Shenton[1], C. Boggis[2], M. Wilson[2], F. Lalloo[1], A. Moran[3], A. Howell[4].* 1) Dept Genetics, St Mary's Hosp, Manchester, United Kingdom; 2) Department of Radiology, Nightingale Breast Screening unit, Withington Hospital, Manchester M20UK; 3) Centre for cancer Epidemiology Christie Hospital Manchester M20, UK; 4) Deartment of Medical Oncology, Christie Hospital, Manchester M20, UK.

Introduction: Accurate individualized breast cancer risk assessment is essential to provide risk-benefit analysis prior to initiating interventions designed to lower breast cancer risk. Several mathematical models for the estimation of individual breast cancer risk have been proposed. However, no single model integrates family history, hormonal factors and benign breast disease in a comprehensive fashion. A new model by Tyrer-Cuzick has addressed these deficiencies. Therefore, this study has assessed the goodness of fit and discriminatory value of the Tyrer-Cuzick model against established models namely: Gail, Claus and Ford. Methods: The goodness of fit and discriminatory accuracy of the models was assessed using data from 1933 women attending the Family History Evaluation and Screening Programme of which 52 developed cancer. All models were applied to these women over a mean follow up of 5.27 years to estimate risk of breast cancer. Results: The ratios of expected to observed numbers of breast cancers (95% confidence intervals [CI]) were 0.48 (0.37-0.64) for Gail, 0.56 (0.43-0.75) for Claus, 0.49 (0.37-0.65) for Ford and 0.81 (0.62-1.08) for Tyrer-Cuzick. The accuracy of the models for individual cases was evaluated using ROC curves. These showed that the area under the curve was 0.735 for Gail, 0.716 for Claus, 0.737 for Ford and 0.762 for Tyrer-Cuzick. Conclusion: The Tyrer-Cuzick model is the most consistently accurate model for prediction of breast cancer. Gail, Claus and Ford all significantly underestimate risk although the accuracy of Claus may be improved by adjustments for other risk factors.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 62 from 2003 ASHG Annual Meeting

Structured assessment of breast cancer risk communication in primary care. *J. Culver*[1,2], *N. Press*[3], *D. Bowen*[1,2], *L. Pinsky*[2], *S. Reynolds*[2], *W. Burke*[1,2]. 1) Fred Hutchinson Cancer Research Center, Seattle, WA; 2) University of Washington, Seattle, WA; 3) Oregon Health and Science University, Portland, OR.

Communication about breast cancer risk represents a challenge for primary care providers, requiring skill in risk assessment and communication. We evaluated primary care providers' skills using unannounced standardized (simulated) patients (SP's) trained to present their concerns and family histories in a consistent way. The study included 86 internal medicine or family practice physicians in the Seattle area who consented to see an unannounced SP. Physicians were randomized to receive one of three cases: Anita (n=25), presenting with anxiety about breast cancer and a transient breast lump, but without a strong family history; Rachel (n=33), presenting with an unrelated problem and expressing concern over her sister's breast cancer but unaware of the significance of her paternal family history of breast and ovarian cancer; and Molly (n=28), presenting with concern about her strong maternal family history of breast cancer. The session's length was measured by audiotape. After the appointment, three qualitative parameters were assessed by SP's on a 3 point scale: whether the physician took adequate time, whether the physician acknowledged her concerns, and whether the physician offered reassurance. The mean level of physician performance associated with Anita's visits (2.92) was significantly higher than that for Rachel's (2.25) or Molly's (2.42) ($p<.0001$). Visits were significantly shorter for both Anita (19.4 min) and Rachel (20.1 min) than for Molly (30.2 min) ($p<.0001$). SP ratings of physician performance were not influenced by the session length or physician gender. We hypothesized that physicians were more familiar with the Anita scenario and more comfortable communicating about her lower risk of breast cancer and were more consistently able to provide her with reassurance and support. Physicians may be more unprepared or uneasy addressing the issues raised by more complex scenarios, particularly in a time-constrained practice. The results suggest that primary care physicians may benefit from additional training in the assessment and communication of breast cancer risk.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Can a computer program replace genetic counseling for BRCA1/2 genetic testing? *M.J. Green*[1], *S.K. Peterson*[2], *M. Wagner Baker*[3], *L.C. Friedman*[4], *G.R. Harper*[5], *W.S. Rubinstein*[6], *D. Mauger*[1]. 1) Penn State College of Medicine, Hershey, PA; 2) UT MD Anderson Cancer Center, Houston, TX; 3) Penn State Cancer Institute, Hershey, PA; 4) Baylor College of Medicine, Houston, TX; 5) Lehigh Valley Hospital, Allentown, PA; 6) Evanston Northwestern Healthcare, Evanston, IL.

As genetic services shift to primary care, new resources for educating patients may be needed. We compared the effectiveness of a computer-based decision aid about BRCA1/2 genetic testing with standard genetic counseling. From 5/00 to 10/02, 211 women with a family or personal history of breast cancer were randomized at 6 study sites to receive genetic counseling alone or education by a computer-based decision aid prior to genetic counseling. Results are reported for measures administered before and after completing the computer or counselor education. Both groups had comparable demographics, experience with computers, medical literacy, and baseline knowledge of breast cancer and genetic testing. Mean age was 44 years, 56% completed college, and 93% were white. The mean knowledge score (on a scale of 0-100) increased in the Counselor Group from 53 before counseling to 82 after ($p<0.0001$), and in the Computer Group from 55 to 91 ($p<0.0001$). The mean knowledge score after computer use was significantly higher than after counseling ($p=0.01$). Mean post-intervention decisional conflict scores (regarding genetic testing decisions) were low in both groups; however, the mean score in the Counselor Group, 1.9 (on a scale of 1-5), was significantly lower than in the Computer Group, 2.1 ($p=0.043$). Further, the Counselor Group had a significantly higher mean score on a scale measuring satisfaction with testing decisions compared with the Computer Group (4.4 and 4.1 respectively, on a scale of 1-5, $p=0.025$). These findings show that use of an interactive computer program may be more effective than standard genetic counseling for increasing knowledge of breast cancer and genetic testing. However, standard genetic counseling was superior for facilitating genetic testing decisions that are less conflicted and more satisfying, suggesting that computer-based decision aids should supplement, rather than replace, genetic counseling.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Adverse effects of Paclitaxel can be precisely predicted by combinations of polymorphisms on two genomic loci.

M. Isomura[1], M. Yoshimoto[2], F. Kasumi[2], M. Matsuura[1], T. Noda[1], T. Muto[1], Y. Miki[1]. 1) Genomecenter, Cancer Institute, Toshima, Tokyo, Japan; 2) Dep. Breast Surgery, Cancer Inst. Hospital, Toshima, Tokyo, Japan.

To predict probability of adverse effects on paclitaxel treatment, we conducted genotyping analysis on breast cancer patients enrolled onto neo-adjuvant weekly paclitaxel therapy. All participants exhibiting almost the same stage of breast cancer were administrated single agent of paclitaxel (80mg/m^2). Adverse effects were evaluated every 7 days and graded according to NCI-CTC grading. To elucidate SNPs associated with granulocytopenia, genotypes of 2,727 SNPs over 298 genes on 54 participants were determined by Invader assay. Associations were examined by comparing genotypes of patients with and without granulocytopenia. The association analyses revealed SNPs on two loci were associated with granulocytopenia ($p = 0.0020$, OR = 10.7, 95% C.I.: 2.09 55.6 and $p = 0.0062$, OR = 5.11, 95% C.I.: 1.41 18.5). In addition, the probability of granulocytopenia on paclitaxel treatment was calculated more precisely by a combination of genotype on two loci. Based on the frequency in Japanese population, the result of our study for prediction of granulocytopenia by paclitaxel treatment will benefit more than 50% of Japanese patients.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 65 from 2003 ASHG Annual Meeting

Role of the large common fragile site gene Parkin in cancer development. *F. Wang, C.L. Hall, S.R. Denison, D.I. Smith.* Div. of Experimental Pathology, Mayo Clinic, Rochester, MN.

Parkin is an extremely large gene (1.7 Mb) which was originally identified as it is mutated in some patients with autosomal recessive juvenile Parkinsonism. Parkin encodes for an E3 ubiquitin ligase and is derived from within 6q26 a chromosomal region that is frequently deleted in multiple tumor types and that also contains FRA6E, a highly active common fragile site. Parkin spans the distal half of the FRA6E region and microsatellite markers within Parkin show the greatest LOH of any 6q26 markers in ovarian tumors. We have detected aberrant Parkin transcripts in multiple tumor types and have identified one hepatocellular carcinoma (HCC) cell line, PLC5, with a homozygous deletion of exon 3. We analyzed ovarian and HCC tumors for mutations in Parkin, but have not identified any point mutations. What was observed was frequent heterozygous deletions as well as duplications of Parkin exons. Western blot analysis has revealed a complete absence of Parkin protein in 9 of 9 HCC cell lines, and decreased or absent Parkin expression in most ovarian cancer cell lines and primary tumors. There are thus many similarities between Parkin and two other extremely large common fragile site genes, FHIT and WWOX. To determine the role that Parkin plays in normal epithelial cells and how its loss contributes to cancer development we have initiated functional studies of Parkin. We generated stable Parkin transfectants into PLC5 and used these to characterize proteins that interact with Parkin using co-immunoprecipitation. This analysis revealed that actin and cytokeratin 18 are bound to Parkin. We have also demonstrated that Parkin binds to keratin in the periphery of cells and that Parkin can specifically ubiquitinate keratin in vitro. When we compared the global gene expression profile changes associated with Parkin re-expression we found that many more genes were down-regulated in response to Parkin when cells were grown in the presence of matrigel. Our results suggest that Parkin is involved in cell-matrix interactions and that the frequently observed loss of expression of Parkin in ovarian and HCC cancers does play an important role in cancer development.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 66 from 2003 ASHG Annual Meeting

A new mechanism of chromosomal instability: inactivation of a protein involved in ribosome biogenesis. *A. Killian, N. Le Meur, R. Sesboue, J. Bourguignon, G. Bougeard, G. Gautherot, C. Bastard, T. Frebourg, J.M. Flaman.* INSERM EMI 9906, Faculty of Medicine, Rouen, France.

Molecular bases of chromosomal instability (CIN), which is observed in the majority of cancers, remain to be characterized. Most cancer cells exhibiting CIN have a defect in their mitotic spindle checkpoint, and genes whose expression is induced when the spindle checkpoint is activated are potentially involved in the control of chromosomal segregation. We show that in yeast inactivation of one of these genes, YMR131c/RRB1, which is involved in early ribosome assembly, alters the spindle checkpoint, chromosomal segregation and blocks the metaphase/anaphase transition. Furthermore, we found in yeast a genetic interaction between RRB1 and ORC6, a gene involved in DNA replication and chromosomal segregation. The human homologue of the Rrb1 protein (Rrb1p) is the GRWD protein and we show that transient depletion of the GRWD protein by small interfering RNA (siRNA) resulted in an increase of abnormal mitoses with appearance of binucleate or hyperploid cells, multipolar spindles and aberrant metaphase plates. Ribosome biogenesis and mitosis are highly coordinated and expression of ribosomal proteins are commonly altered in primary tumours. The Rrb1 protein could be one of the molecular links between ribosome biogenesis and mitosis, its inactivation leading to an chromosomal instability. These results indicate that inactivation of the Rrb1p pathway involved in early ribosome biogenesis alters chromosome segregation, and thus reveal a new potential mechanism for CIN.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 67 from 2003 ASHG Annual Meeting

Molecular classification of endometrial cancers identifies women who are at increased risk for genetic disease. *P. Goodfellow, B. Buttin, S. Babb, D. Mutch.* Washington Univ Medical Ctr, St Louis, MO.

There are approximately 40,000 new endometrial cancers (EC) each year in the US. Most are sporadic. However, up to 5% of EC are inherited. At present, identification of women with inherited EC is largely limited to cases that fulfill the criteria for clinical diagnosis of hereditary nonpolyposis colorectal cancer (HNPCC). More effective means of identifying women with HNPCC and/or inherited defects in DNA mismatch repair are needed. In this study we demonstrate that molecular classification of EC reveals a subset of cases with earlier onset disease, increased risk for synchronous or metachronous HNPCC-associated cancers and positive family history.Microsatellite instability (MSI) is a feature of tumors with defective DNA mismatch repair. Approximately 28% of EC have MSI. MSI+ EC are usually sporadic and associated with methylation of the MLH1 promoter (Esteller et al., 1998; Simpkins et al., 1999; Goodfellow et al., 2003). We previously demonstrated that women with MSI+ EC in which the MLH1 is unmethylated (MSI+U) frequently harbor inherited mutations in DNA mismatch repair. In a series of 35 cases with MSI+U EC we identified 7 MSH6 and 5 MSH2 mutations (Goodfellow et al., 2003). In this study we assessed family history and the frequency of synchronous and metachronous cancers in women with MSI+U EC and compared the findings with those for women with MSI- EC and MSI+ EC with MLH1 methylation. Rates of synchronous and metachronous cancers were similar in the MSI+ and MSI- groups at 20 and 23%, respectively. However, patients with MSI+U EC (N=28) had an excess of HNPCC-associated second and third cancers compared to those with MSI+ MLH1 methylated (N=66) and MSI- EC (N=94) (18% versus 4.5%, P=0.034, and 2.1%, P=0.002). Women with MSI+U EC were younger than the rest of the patient population (P<0.001) and more likely to have a family history of cancers. Taken together, these data suggest that women with MSI+U EC are at increased risk for inherited cancer susceptibility. Analysis of tumors may prove useful in the identification of patients and families that would benefit from more intense cancer surveillance.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

The ataxia-telangiectasia protein ATM reacts rapidly to *in vivo* DNA damage. *M.S. Meyn[1,2], D. Young[3], K.K. Khanna[3], W. Wang[1]*. 1) Genetics and Genomic Biology, Hosp Sick Children, Toronto, Canada; 2) Molec & Med Genetics and Paediatrics, Univ of Toronto, Toronto, Canada; 3) Queensland Inst for Medical Research, Brisbane, Australia.

We are studying in vivo interactions between chromosome instability syndrome proteins and double-stranded DNA breaks (DSBs) induced by laser micro-irradiation. Using anti-ATM antibodies, we find human fibroblasts accumulate ATM protein in DSB-containing regions of their nuclei within 5 min post-irradiation. These ATM foci persist for >6 hours, even though most DSBs are repaired within 2 hours. There is a high degree of co-localization between DSB-containing chromatin and foci of ATM, FANCD2, BRCA1, and BLM, while H2AX foci are more diffusely distributed. Association of ATM with DSBs does not require NBS1, BRCA1, BLM or FANCD.

To clarify early events we have studied the in vivo behavior of a GFP-tagged ATM that complements the phenotype of ataxia-telangiectasia cells. We find most of the GFP-ATM is rapidly diffusible within unirradiated nuclei of transfected fibroblasts. Accumulation of GFP-ATM at DSBs can be detected within 3 seconds post-irradiation, plateaus by 1 minute and remains stably elevated for at least 20 minutes. The residence time for GFP-ATM is prolonged in nuclear regions containing DSBs. The kinetics of DSB association are normal for GFP-ATM S1981A, a mutant that cannot be phosphorylated at serine 1981. In contrast, a kinase deficient GFP-ATM mutant shows impaired accumulation and retention at sites of DSB damage.

Our findings support the idea that ATM acts as a sensor/trigger for DNA damage response network(s). The localization of ATM to DSBs occurs more slowly than predicted by simple diffusion, indicating that additional steps may be required for localization to DSBs. The increased residence time of GFP-ATM at DSB sites suggests that GFP-ATM may stably associate with DSB-containing chromatin. Our results suggest a model in which ATM phosphorylation of itself, and perhaps other proteins, is not required for initial association of repair proteins with DSBs but acts to stabilize interactions of repair proteins with each other and/or with damaged chromatin at DSB sites.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Heterozygosity for p53 (*Trp53*-/+) Accelerates the Tumor Phenotype of Fanconi Anemia Complementation Group D2 (*Fancd2*) Knockout Mice. *S. Houghtaling[1], M. Finegold[2], M. Grompe[1]*. 1) Molec & Med Genetics, SOM Oregon Hlth & Sci Univ, Portland, OR; 2) Dept of Pathology, Texas Children's Hospital, Baylor College of Medicine, Houston, TX.

Fanconi anemia (FA) is an autosomal recessive disease characterized by progressive bone marrow failure and an increased susceptibility to cancer. The disease is genetically heterogeneous, consisting of at least 8 complementation groups, FA-A through G, including FA-D1 and D2. The genes for 6 of these have been cloned and recently inactivation of *BRCA2* was shown to cause FA in patients from complementation groups B and D1. We have previously reported an increased incidence of epithelial cell tumors in *Fancd2* knockout mice. To further investigate the role of the FA pathway in tumor prevention, we generated a cohort of *Fancd2* mutant and control mice in the 129 background strain that are heterozygous for the tumor suppressor gene, *Trp53*. *Trp53* heterozygous mice have previously been shown to develop lymphomas and sarcomas at 10 to 14 months of age. To date, 10 of 20 *Fancd2*-/-, *Trp53*-/+ mice have developed neoplasms. In addition, the onset of tumors in *Fancd2* mutants was decreased from ~13.5 months to ~10 months when accompanied by heterozygosity at *Trp53*. The tumor spectrum in *Fancd2*-/-, *Trp53*-/+ mice included sarcomas that are commonly observed in *Trp53* heterozygotes, as well as mammary adenocarcinomas that occur rarely in *Trp53* heterozygotes. We are currently investigating the genetics of the tumors, specifically whether loss of heterozygosity at the *Trp53* locus contributed to the phenotype. Our preliminary results suggest that *Fancd2* and *Trp53* act cooperatively in the prevention of mammary adenocarcinoma in the 129 strain, thus confirming our previous finding that the FA pathway functions in tumor prevention in *Fancd2* mutant mice.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

SiRNA Depletion of Blooms Helicase Produces Genomic Instability. *A.W. Hemphill, Y. Cox, Y. Akkari, S. Olson, R.E. Moses.* Molecular and Medical Genetics, Oregon Health & Science Univ, Portland, OR.

Blooms syndrome (BS) is a rare recessive disorder characterized by growth defects, immunodeficiency and an increased risk of cancer, and BS cell lines show high levels of sister chromatid exchange (SCE). *BLM*, the gene mutated in BS patients, encodes a member of the RecQ helicase family. These helicases have been suggested to act in replication re-start following DNA damage. Consistent with a role in DNA repair, BLM interacts with known DNA repair proteins, including the MRE11/RAD50/NBS1 complex, RAD51, and MLH1. BLM also co-purifies from cell extracts with RPA and TOPO III, as well as the core Fanconi anemia (FA) protein-complex. To study the function of BLM, we used siRNA to deplete the BLM protein. We found increased chromosomal breaks and quadriradial formation following treatment with mitomycin C (MMC), a DNA interstrand cross-linking (ICL) agent, in wild-type cells transfected with the BLM siRNA. Since the findings of chromosomal instability were like those of FA, we studied the relationship between BS and the FA pathway. We depleted BLM in FA cells and found that BLM appears to be epistatic with FA for ICL repair, since the siRNA for BLM caused no increase in MMC effects. Defects in the FA pathway have been suggested to result in failure to repair double-strand breaks in S-phase, and our findings are consistent with BLM being a part of that repair response.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Short-Term Survival Following High-dose Chemotherapy and Bone Marrow Transplant and Association with a Triplet Repeat in the Glutamate Cysteine Ligase Catalytic Subunit Gene. *A. Kallianpur[1], A.S. Willis[2], B.W. Christman[3], S.R. Summar[4], M.L. Summar[4].* 1) Medicine, Vanderbilt Univ Med Ctr, Nashville, TN; 2) Molecular Physiology and Biophysics, VUMC, Nashville, TN; 3) Pulmonary Medicine, VUMC, Nashville, TN; 4) Pediatric Genetics, VUMC, Nashville, TN.

Cytotoxic therapy regimens in preparation for bone marrow transplant can have many complications, such as hepatic veno-occlusive disease (HVOD) and acute lung injury. These regimens have been associated with increased formation of reactive oxygen species and the depletion of glutathione as well as other antioxidants. Glutamate cysteine ligase (GCL) catalyzes the first, rate-limiting step in the de novo synthesis of glutathione. We have identified 11 polymorphisms in the exons and the intronic flanking regions in the GCL catalytic subunit gene. We genotyped 137 patients undergoing bone marrow transplant for each of the polymorphisms and used univariate and multivariate statistical analysis to determine if complications of the bone marrow transplant, HVOD and death by day 60, were associated with these polymorphisms. No significant associations were found in univariate or multivariate analysis for the development of HVOD. Specific genotypes of the 5 UTR trinucleotide repeat were found to be associated with increased relative risk, RR for 8/9 was 2.21 (95% CI=0.66, 7.4; p=0.24) and for 9/9 was 2.11 (95% CI=0.93,4.82; p=0.08). Multivariate analysis with adjustments for several factors revealed the 7/7 genotype for the GAG repeat was highly protective against early death; the relative risk was 0.037 (95% CI=0.003,0.49; p=0.012). These results indicate that genotype for the triplet repeat in the 5 UTR in GCLC may be an important factor in survival following high-dose chemotherapy and bone marrow transplant.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Evidence for Prezygotic Trinucleotide Repeat Instability at Two Loci: CTG18.1 and ERDA1. *M. Schalling[1], Q.P. Yuan[1], N. Pedersen[2], M. Jansson[2], K. Lindblad-Toh[3], R. Adolfsson[4].* 1) Neurogen Unit, Ctr Molec Med, Karolinska Hosp, Stockholm, Sweden; 2) Department of Medical Epidemiology, Karolinska Institutet, Sweden; 3) Whitehead Institute Center for Genome Research, Whitehead Institute; 4) 4Department of Clinical Sciences, Division of Psychiatry, Ume University, Sweden.

Intergenerational expansions of trinucleotide repeats cause a number of neuromuscular disorders. How and when expansion occurs is largely unclear. Two loci, ERDA1 at17q21.3 and CTG18.1 at 18q21.1, contain polymorphic CAG/CTG repeats that are frequently expanded in the general population. We have studied repeat expansions at these two loci in parent-offspring pairs of families as well as in monozygotic twin pairs, attempting to understand the timing of repeat size change as well as factors influencing repeat stability. We observed intergenerational instability of both repeats upon transmission. Fifty-three percent (27/51) of parent-offspring pairs with ERDA1 expansions displayed repeat instability, and 67% (8/12) of the pairs with CTG18.1 expansions were unstably transmitted. Contractions were more frequent than expansions upon paternal transmission at both loci. Repeat expansions were detected in 28/101 MZ twin pairs (ERDA1) and 6/101 pairs (CTG18.1). No size difference was observed within any of the MZ twin pairs, suggesting that the repeat size changes predominantly occur as a prezygotic event at both loci. A reproducible smearing of gel bands corresponding to the expanded allele was present in 61% (22/36) of the individuals with a CTG18.1 expansion. Small pool PCR of samples with such smears showed multiple allele sizes suggesting that the smear represents somatic mosaicism. Within all MZ twin pairs and most parent-offspring pairs there was complete concordance for the presence or absence of mosaicisms, suggesting that genetic components acting in cis may regulate mitotic instability. However, two parent-offspring pairs with similar size expansions were discordant for the presence of somatic instability, suggesting that additional factors may influence repeat instability in mitosis.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

A new player in X-linked mental retardation. *K. Freude[1], K. Hoffmann[1], L. Jensen[1], V. desPortes[2], H. Yntema[3], B. Moser[1], S. Lenzner[1], S. Schweiger[1], C. Moraine[4], J.P. Fryns[5], J. Chelly[6], B. Hamel[3], H. Scherthan[1], H.H. Ropers[1], V.M. Kalscheuer[1].* 1) Max-Planck-Institute for Molecular Genetics, Ihnestrasse 73, D-14195 Berlin, Germany; 2) Pediatric Deparment, Centre Hospitalier Lyon Sud, Hospices Civils de Lyon, France; 3) Department of Human Genetics, University Medical Centre, Nijmegen, The Netherlands; 4) Services de Genetique -INSERM U316, CHU Bretonneau, Tours, France; 5) Center for Human Genetics, Clinical Genetics Unit, Leuven, Belgium; 6) Institut Cochin de Genetique Moleculaire, CNRS/INSERM, CHU Cochin 75014 Paris, France.

Non-syndromic X-linked mental retardation (XLMR) is a highly heterogeneous condition, and most of the relevant genes are still unknown. We have recently shown that 1/3 of the missing mutations cluster on the proximal Xp and in the pericentric region (Ropers et al, Trends Genet. 19: 316, 2003). In the course of large-scale mutation screening involving brain-expressed genes from a 7.5 Mb Xp11 segment (see abstract Jensen et al, this issue), we have identified a novel MRX gene in the previously decribed MRX44 family. All affected males of this family carry a splice site mutation resulting in skipping of one exon, as shown by RT-PCR experiments on RNA from lymphoblastoid cell lines. Overexpression experiments of the mutant protein, which lacks the skipped exon, showed that its subcellular localization is significant different from that of the full-length protein. Subsequent mutation analyses identified another mutation in a family with non-specific MRX, resulting in a stop mutation. Northern blot analysis of polyA+ RNA from patient and controls showed that this stop mutation is associated with mRNA instability due to nonsense-mediated RNA decay. We currently test additional XLMR families for mutations in this new MRX gene. Its gene product is homologous to three known yeast proteins, and for all three of these, mutant yeast strains are available. These mutant strains are presently being used for complementation studies to identify the yeast ortholog of the newly identified human MRX gene and to determine its func.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

The gene mutated in ataxia-oculomotor apraxia 2 (AOA2) encodes a new RNA/DNA helicase. *M.C. Moreira[1], S. Klur[1], M. Watanabe[2], J.C. Moniz[3], I. Le Ber[4], P. Coutinho[5,6], C. Tranchant[7], J.M. Warter[7], J. Sequeiros[5], A. Brice[4], M. Koenig[1]*. 1) IGBMC - CNRS, INSERM, ULP, Illkirch/Strasbourg, France; 2) Gunma University School of Medicine, Maebashi, Japan; 3) Hospital Sto. António dos Capuchos, Lisboa, Portugal; 4) INSERM U289, Hôpital de la Salpêtrière, AP-HP, Paris, France; 5) UnIGENe - IBMC, ICBAS, Universidade do Porto, Porto, Portugal; 6) Hospital São Sebastião, Sta. Maria da Feira, Portugal; 7) Hôpital Universitaire de Strasbourg, Strasbourg, France.

Ataxia-oculomotor apraxia (AOA) is an autosomal recessive disorder characterized by cerebellar ataxia, ocular apraxia, early areflexia, late peripheral neuropathy, slow progression, severe motor handicap, and the absence of telangiectasias and of immunodeficiency. Non-allelic heterogeneity in AOA was recently demonstrated by the identification of two loci, AOA1 and AOA2, and additional families linked to none of the two. AOA1, located in 9p13, is characterized by an age of onset usually between 2 and 6 years, late appearance of hypoalbuminemia and is caused by mutations in the aprataxin gene. AOA2 is located in 9q34. The study of a large number of families from different countries allowed us to infer that AOA2 is clinically characterized by an onset age between 11 and 22 years and high levels of alpha-fetoprotein in most cases. We localized the defective gene in a 1 Mb critical interval containing 13 genes. Systematic sequencing of the coding sequences revealed disease-causing mutations in a gene encoding for a new member of the RNA/DNA helicase family of proteins. We identified eight different mutations, 7 of which cause premature termination of the protein. Knowing that several recessive ataxias are caused by mutations in genes implicated in DNA repair, such as the ATM, MRE11, aprataxin and tyrosyl-DNA phosphodiesterase 1 genes, it is tempting to speculate that the AOA2 helicase is similarly implicated in DNA repair, as it would strengthen a new pathological paradigm shared among the recessive ataxias defined by primary cerebellar degeneration and may open new therapeutic avenues.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 75 from 2003 ASHG Annual Meeting

Juxtaposed mono- and dinucleotide tracts form a new type of sequence polymorphism.
T.W. Hefferon, G.R. Cutting. Inst Genetic Medicine, Johns Hopkins Univ.

Simple sequence repeats are common throughout the human genome, and are extremely useful in genetic mapping studies because of their highly polymorphic nature. The most common mononucleotide repeat is T_n, and the most common dinucleotide repeat is $(TG)_m$. We have discovered that when found together these two repeats combine to form a sequence element that varies primarily in content rather than length, apparently due to an unusual mutational mechanism. The splice acceptor of intron 8 (IVS8) of the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) contains a polymorphic TG tract (TG_{9-13}), followed by a variable T tract (T_5, T_7, T_9). Variation in each of these tracts influences exon 9 splicing and modifies diseases status. Interestingly, not only does the T-tract vary strictly by two-basepair increments, but there is an inverse relationship between the lengths of the two sequence elements. Thus while there are theoretically fifteen allele combinations ranging in size from 23 to 35 bp, only eight of these have been observed, all between 27 and 31 basepairs long. In addition, others have reported a rare $TG_{13}T_3$ variant and we have found a rare TG_9T_{11} variant, both of which fall within the described size range and are additional examples of the inverse relationship between the two repeat tracts. Sequencing of the *CFTR* IVS8 locus in 20 chimpanzees revealed two alleles, TG_8T_9 and TG_9T_7, which conform to characteristics of the TG_mT_n tract observed in human *CFTR*. To determine whether the sequence TG_mT_n is inherently susceptible to this form of variation, we performed a BLAST search of the human genome for TG_5T_5 and found 3200 examples. Sequencing of eighteen individuals from diverse genetic backgrounds for five of these loci revealed variation in poly-T tract length in 2-basepair increments, as well as restricted total TG_mT_n tract length. Thus the relatively common juxtaposition of TG and T tracts creates a previously unrecognized sequence element that displays a novel form of variation.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 76 from 2003 ASHG Annual Meeting

TNFA gene polymorphisms in glucose homeostasis and adiposity: The IRAS Family Study. *B.S. Sutton[1,2], S. Weinert[1,2], C.D. Langefeld[1,4], S. Beck[1,4], J.M. Norris[5], N.D. Palmer[1,2], D.W. Bowden[1,2,3].* 1) Wake Forest University School of Medicine, Winston Salem, NC; 2) Center of Human Genomics; 3) Department of Internal Medicine; 4) Department of Public Health Sciences; 5) Department of Preventive Medicine and Biometrics University of Colorado Health Sciences Center Denver, CO.

The TNF-alpha protein has been implicated in insulin resistance and obesity. We hypothesized that TNFA polymorphisms are positively associated with glucose homeostasis and adiposity. We have evaluated four TNFA gene promoter polymorphisms, G-238A, G-308A, G-376A, and C-863A in 1049 DNA samples from 63 African American and Hispanic families participating in the IRAS Family Study and evaluated their association with measures of glucose homeostasis and adiposity. Allele frequencies of the SNPs were similar between the ethnic groups. In the combined analysis of both ethnic groups the G-308A polymorphism was significantly and positively associated with HOMA index of insulin resistance (P=0.005), fasting insulin (P=0.006), and acute insulin response (AIR; P=0.033), but was not associated with insulin sensitivity (S_i), or glucose effectiveness (S_g). The G-238A, G-376A, and C-863A SNPs were not associated with any glucose homeostasis traits in the overall analysis. In African American subjects, all measures of glucose homeostasis were significantly associated with G-308A: S_i(P< 0.00001); S_g (P<0.0003); AIR (P< 0.00009); HOMA index (P=0.001); fasting insulin (P=0.009). In addition, the G-238A SNP showed evidence of association: S_i, S_g, DI, AIR, fasting glucose (all P< 0.00001) and fasting insulin (P=0.028). Analysis of the combined ethnic groups revealed evidence of significant association between measures of adiposity and the G-308A SNP: body mass index (P=0.007); waist-to-hip ratio (P=0.02); visceral (P=0.009) and subcutaneous adipose tissue (P=0.036) as measured by computed tomography. Again the G-238A, G-376A, and C-863A SNPs were not associated with adiposity traits in the overall analysis. These results for G-308A are consistent with earlier data that suggest the SNP is functional and may affect measures of glucose homeostasis and adiposity.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 77 from 2003 ASHG Annual Meeting

IL-13 gene sequence variants and haplotypes are associated with asthma, bronchodilator responsiveness and asthma Severity in Mexicans and Puerto Ricans from the Genetics of Asthma in Latino Americans (GALA) study. *S. Choudhry[1], D.L. Lind[1], K. Salari[1], N. Ung[1], N.E. Coyle[1], E. Ziv[1], S.L. Clark[1], S. Nazario[2], J.R. Rodriguez-Santana[3], J. Salas[5], M. Selman[5], H.A. Boushey[1], S.T. Weiss[4], R. Chapela[5], J.G. Ford[6], W. Rodriguez-Cintron[2], P. Kwok[1], E.K. Silverman[4], D. Sheppard[1], E.G. Burchard[1]*. 1) University of California, San Francisco, CA; 2) University of Puerto Rico, San Juan, San Juan, Puerto Rico; 3) Pediatric Pulmonary Program of San Juan, Cardiovascular Center of Puerto Rico, San Juan; 4) Brigham and Womens Hospital, Boston, Massachusetts; 5) Instituto Nacional de Enfermedades Respiratorias (INER), Mexico City, Mexico; 6) Harlem Lung Center, Harlem Hospital and Columbia University, New York, New York.

In the U.S., Puerto Ricans and Mexicans have the highest and lowest asthma prevalence, morbidity and mortality, respectively. Although there are many potential explanations for this observation, including environmental and socioeconomic factors, one potential explanation is that the genetic predisposition to asthma differs among subgroups within the Latino American population. To investigate this the Genetics of Asthma in Latino Americans (GALA) Study was established.We identified SNPs by sequencing the IL-4 and IL-13 genes in 72 individuals from three asthmatic populations: Mexican, Puerto Rican and African Americans. We then used the TDT to test for association between these SNPs and their haplotypes to asthma, bronchodilator responsiveness and asthma severity among 583 family trios participating in the GALA Study. 31 SNPs were identified, of which 8 (26%) were novel. LD patterns and haplotype frequencies were substantially different among the three populations. IL-13 but not IL-4 SNPs and haplotypes were strongly associated with asthma, asthma severity and bronchodilator responsiveness among Puerto Ricans and less so among Mexicans ($p = 0.03$-0.0003). These results demonstrate that there are ethnic-specific differences in genetic risk factors for asthma, asthma severity and drug response. The striking differences in asthma burden and drug response among Mexicans and Puerto Ricans merits further investigation.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

A Linkage Study In Heavy Smokers Suggests The Presence Of Both Causative And Protective Genes For Chronic Obstructive Pulmonary Disease (COPD). *H. Hakonarson[1], E. Halapi[1], A. Sigvaldason[2], H. Jonssson[1], T. Gislason[2], J. Laufs[1], L. Thorsteinsson[1], E. Soebeck[1], V. Gudnason[3], H. Andrason[1], S. Oskarsson[2], M. Frigge[1], A. Kong[1], J. Gulcher[1], K. Stefansson[1].* 1) deCODE genetics, Inc, Reykjavik, Iceland; 2) Department of Pulmonary Medicine, National University Hospital, Reykjavik, Iceland; 3) Icelandic Heart Association, Reykjavik, Iceland.

COPD occurs in smokers and is characterized by chronic progressive airflow limitation encompassing pulmonary emphysema and chronic bronchitis. Tobacco smoke is in general necessary but not sufficient for the development of COPD. Indeed, only 15% of chronic smokers develop COPD. This information, together with evidence from both familial and twin studies suggests that smokers susceptibility to developing COPD is genetically determined. By leveraging the genealogy database covering the entire Icelandic nation we identified 166 COPD families with 466 patients and 149 families that included 461 heavy smokers without COPD. Linkage analysis of these 466 COPD patients and 540 of their unaffected relatives demonstrated genome-wide significant (GWS) linkage on chr 2p and 3q, with allele-sharing lod scores of 4.1 and 3.7, respectively ($p < 2x10-5$). A lod score of 3.4 was observed on chr 10q. In contrast, the 461 heavy smokers without COPD demonstrated linkage on chr 7q with allele sharing lod score of 2.6, with no evidence of genetic sharing on chr 2p, 3q or 10q. Of note, emphysema and chronic bronchitis co-segregated within most (73%) of the COPD families. Taken together, this study demonstrates that heavy smokers with COPD map to chr 2p, 3q and 10q, whereas heavy smoker without COPD map to chr 7q suggesting the presence of both causative and protective genes for COPD. The co-segregation of emphysema and chronic bronchitis within most of the COPD families, together with significant linkage results support the notion that emphysema and chronic bronchitis share major genetic factors. In view of the latter, we speculate that the COPD genes we have mapped may have a large variation in expressivity, the basis of which may be interacting genes or variation in exposure to smoke or other environmental factors.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 79 from 2003 ASHG Annual Meeting

Meta-analysis of 11 genome scans for cleft lip with or without cleft palate. *M.L. Marazita[1], J.C. Murray[2], M. Cooper[1], T. Goldstein[1], R. Schultz[2], S. Daak-Hirsch[2], L. Field[3], Y. Liu[1], G. Tunçbilek[1], A. Ray[3], N. Prescott[4], R. Winter[4], D. Wyszynski[5], J. Bailey-Wilson[6], H. Albacha-Hejazi[7], A. Lidral[2], L. Moreno[2], M. Arcos-Bargos[8], T. Beaty[9].* 1) U of Pittsburgh/Zhabei Institute, China/Hacettepe U, Turkey; 2) U of Iowa; 3) U of B.C./U of Toronto, Canada; 4) Inst of Child Health, London; 5) Boston U; 6) NHGRI; 7) Ibn Al-Nafees Hosp, Syria; 8) U of Antioquia, Colombia; 9) Johns Hopkins U.

Despite many genetic studies of nonsyndromic cleft lip with or without cleft palate (NSCL/P) progress has been slow with results varying between studies. To identify genetic regions likely to include CL/P susceptibility loci, we performed a meta-analysis of 11 genome scans (568 multiplex families, 3543 genotyped individuals): 4 scans published or in press (England, Prescott et al; China, Marazita et al; Syria, Wyszynski et al; Turkey, Marazita et al), 4 presented at this meeting (Philippines, Schultz et al; Pennsylvania, Weinberg et al & Neiswanger et al; Colombia & Ohio, Moreno et al), and 3 recently completed (Turkey, China, India). We used the Genome Scan Meta-Analysis method (GSMA, Wise et al) that allows the combination of genome-scan results from studies with differing markers, statistical analyses, and/or family types. The genome was divided into 124 30-cM bins. In each study each bin was ranked according to the bin's best linkage result, bin ranks were summed over all studies and GSMA p-values determined. 10 bins representing 6 chromosomes had p-values <0.05. Notable results supporting previous reports with NSCL/P include 1q22.3-41 (p=0.02/0.03; 2 adjacent bins, IRF6 region-see Zucchero et al, this meeting), 2p13 (p=0.003, region with TGFA), 6p21.3-21.1 (p=0.01), 17q12 (p<0.001, region with RARA). Novel regions identified include 2q35-36 (p<0.0001), 7p13-15 (p=0.004), 7q22-qter (p=0.03/0.04; 2 adjacent bins), 12q24-qter (p=0.03). Thus, this first meta-analysis of NSCL/P identified multiple regions to focus fine mapping and candidate gene identification efforts. NIH grants DE-09886, DE-12472, DE-08559, DE-13076, DE-14667, RR-00084; UK Action Research, Birth Defects Foundation; Mammalian Genotyping Service; Center for Inherited Disease Research.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 80 from 2003 ASHG Annual Meeting

Quantitative Trait Locus Analyses of Pooled Genome Scans Identify Loci Affecting Human Stature, Body Mass Index and Serum Lipid Levels. *S.A. Sammalisto[1], K. Sood[1], T. Hiekkalinna[1], M.R. Taskinen[2], T. Tuomi[2], L.C. Groop[3], L. Peltonen[1], M. Perola[1].* 1) Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 2) Department of Medicine, Helsinki University Central Hospital; 3) Department of Endocrinology, Wallenberg Laboratory, University Hospital MAS, Lund University, Malmo, Sweden.

Data on height, body mass index (BMI), and serum lipids are frequently collected in conjunction with genome-wide scans designed for different phenotypes, but often used merely for adjustment or ascertainment purposes. We have pooled the raw genotype and phenotype data from four Finnish genome scans independently ascertained for Familiar Combined Hyperlipidemia, low-HDL and type II diabetes (DMII) with a total of 3770 individuals from 457 families and performed linkage analyses for stature, BMI, High-density lipoprotein cholesterol (HDL), total cholesterol (TC) and triglyceride (TG) concentrations. Our strategy was first to build a merged, well-ordered marker map across the studies using the April 2003 release of the human genome sequence and interpolating the genetic map using the published DeCode map markers as anchoring points. Subsequently the marker information from different scans was combined and we performed linkage analysis with the pooled dataset for each phenotype with the proper covariates using variance component analysis (MERLIN). For stature, two previously reported genomic regions showed suggestive evidence of linkage: 22q11 (D22S446, LOD=2.67) and 6p25 (D6S1574 LOD= 2.10). Interestingly, both BMI (D15S657, LOD=2.86) and cholesterol (D15S1515, LOD=2.54) showed suggestive linkage to 15q26, which harbours several interesting candidate genes. A region previously linked to BMI in several other studies, 16p12 was also detected in our study (D16S769, LOD=2.30). The best evidence for linkage was found between HDL and a locus on 18p11 (D18S873, LOD=3.32), previously linked to a high-BMI subgroup of type II diabetic patients. Suggestive linkage for HDL was also found to 5p14 (D5S1473, LOD=2.14) and for TG to 3q29 (D3S1265, LOD=2.19). Our results demonstrate the value of pooled data analyses in identification of human QTLs.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Genome-wide association mapping in a founder population identifies *ITGB3* as a QTL for whole blood serotonin levels.
L. Weiss[1], J. Veenstra-VanderWeele[2], M. Abney[1], D. Newman[1], H. Dytch[1], M.S. McPeek[3], S. Cheng[4], E. Cook Jr.[1,2], C. Ober[1]. 1) Human Genetics, U Chicago, Chicago, IL; 2) Psychiatry, U Chicago; 3) Statistics, U Chicago; 4) Roche Molecular Systems, Inc., Alameda, CA.

Serotonin has vital roles in the cardiovascular, intestinal and nervous systems and has been studied as a peripheral correlate in psychiatric phenotypes. The few ancestral genomes and extensive LD present in the Hutterites, as well as a homogeneous environment resulting from their communal lifestyle, make the Hutterites an ideal population in which to map quantitative trait loci (QTLs). We conducted genome-wide association and linkage mapping studies to identify QTLs for serotonin levels in the Hutterites. Whole blood serotonin was measured by HPLC in 567 Hutterites unselected for any phenotype. These individuals were genotyped for 1,123 markers: 884 STRPs, 239 intragenic polymorphisms. Pedigree-based tests developed for QTL mapping in large pedigrees (Abney et al., 2002, Am J Hum Genet 70:920-34), include a linkage method, homozygosity-by-descent (HBD), and an association method, allele-specific HBD (ASHBD), and utilize the probability of HBD at a locus or for an allele, respectively, as a predictor of a quantitative trait value. Significance was assessed by a permutation test. The most significant association was a SNP (Leu33Pro) in integrin 3 (*ITGB3*) at 66 cM on chr 17 (LOD = 3.3, $P = 9.75 \times 10^{-5}$). By HBD linkage mapping, a modest peak on chr 17 at 70 cM was also observed (LOD = 1.87, $P = 0.0033$). When genotype for *ITGB3* Leu33Pro was included as a covariate in the linkage analysis, the linkage peak was nearly completely eliminated (LOD = 0.02, $P = 0.76$ at 67 cM). This indicates that either this SNP or a polymorphism in strong LD with it is responsible for the linkage signal in this region. In fact, further studies of variation in this gene have identified a silent SNP that shows more significant association with serotonin levels, indicating that additional variation may be relevant. The association of serotonin levels with a novel candidate, *ITGB3*, and the fact that it accounts for nearly all the evidence of linkage in the region suggest that this gene may be an important serotonin QTL.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 82 from 2003 ASHG Annual Meeting

Interaction of loci on chromosomes 7 and 10 influences blood pressure. *X. Zhu*[1], *X. Wu*[1], *A. Luke*[1], *A. Adeyemo*[2], *D. Kan*[1], *N. Bouzekri*[3], *R. Cooper*[1]. 1) Preventive Med & Epidemiology, Loyola Univ Medical Ctr, Maywood, IL; 2) Department of Pediatrics/Institute for Child Health, College of Medicine, University of Ibadan, Ibadan, Nigeria; 3) Department of Cardiovascular Medicine, University of Oxford, The Wellcome Trust Centre for Human Genetics, Oxford, UK.

Genetic effects may be important in determining susceptibility to hypertension since as much as one-third of the variance of BP is due to heritable influences. To date, however, there is no convincing evidence that supports specific genetic variants as a cause of hypertension. It has been suggested that several quantitative trait loci and their interaction may account for the BP variation. In this study, 249 large families were recruited from southwest Nigeria, and 378 microsatellite markers were genotyped. Multipoint variance component analysis identified marginal linkage evidence to SBP and DBP on 7 and 6 chromosomal regions, respectively (LOD>1.5). We then calculated the pairwise correlations of the IBD sharing between these regions for independent affected sib pairs. The family-specific nonparametric linkage (NPL) scores were also computed. Both the affected sib pair allele-sharing probability and the family-specific NPL score demonstrated that there were positive correlations between 7q21-31 and 10p14-12 for both SBP and DBP. By conditioning on the linkage evidence at 7q21-31, the LOD score at 10p increased from 1.64 in the baseline analysis to 2.78 for SBP, and 1.86 to 3.23 for DBP. The results suggest that epistatic interactions between the regions on chromosomes 7 and 10 may attribute the hypertension variation.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Autosomal dominant and recessive anhidrotic ectodermal dysplasia are allelic diseases at the EDARRAD locus.

L. Baala[1,2], F. El-Kerch[2], S. Hadj-Rabia[1], A. Munnich[1], S. Lyonnet[1], A. Sefiani[2], A. Smahi[1]. 1) INSERM-U393 and Service de dermatologie, Hopital Necker, Paris, France; 2) Department of Medical Genetics, INH, Rabat, Morocco.

Anhidrotic ectodermal dysplasia (EDA) are disorders of ectodermal differenciation characterized by sparse hair, abnormal or missing teeth and unability to sweet. The X-linked EDA is the most common form, ascribed to mutations of the EDA gene that encodes ectodysplasin, a member of the TNF family. Autosomal dominant and recessive forms of EDA, indistinguishable from the X-linked variant, have been also described. Two different disease genes have been hitherto identified. Mutations in EDA-3 encoding EDAR, a TNF receptor, result in either dominant or recessive forms. In addition, mutations in a recently identified gene, EDARADD (Edar-Associated Death Domain) have also been shown to result in recessive EDA.We report on a large moroccan family with an autosom al dominant EDA. The 8 affected individuals presented with hypotrichosis, hypodontia, and anhidrosis. Genetic analysis showed linkage to chromosome 1q42-q43 where the EDARADD gene has been mapped. We screened the 7 exons of this gene and found a novel mis sense mutation (G335-->T) which changed a leucine residue into an arginine (Leu 112-->Arg). We demonstrated that this mutation impairs NF-KB activation. Thus the EDARADD gene accounts for both recessive and dominant EDA. EDAR is activated by its ligand, e ctodysplasin and uses EDARADD to build an intracellular complex that, in turn, activates NF-kB. The missense mutation reported here probably disrupts the interaction of EDARADD with EDAR and impairs the dowstream signaling pathway which plays a crucial ro le in the differentiation of skin appendages. This data might explain the phenotypic similarity of the X-linked and autosomal forms of anhidrotic ectodermal dysplasia.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Strong association of intragenic SNPs of the 6q25 region with leprosy in two independent samples. *M. Mira*[1], *A. Alcais*[2], *N. Thuc*[3], *M. Moraes*[5], *C. Di Flumeri*[1], *N. Martin*[1], *V. Thai*[3], *N. Huong*[3], *A. Verner*[4], *P. Lepage*[4], *A. Montpetit*[4], *T. Hudson*[4], *L. Abel*[2], *E. Schurr*[1]. 1) Dept Biochemistry, McGill Univ, Montreal, Canada; 2) INSERM U.550, Paris, France; 3) Dermato-Veneorology hospital, Ho Chi Minh City, Vietnam; 4) McGill University and Genome Quebec Innovation Centre, Montreal, Canada; 5) Dept Tropical Medicine, IOC-Fiocruz, Rio de Janeiro, Brazil.

Leprosy is a chronic infectious disease caused by Mycobacterium leprae and 700,000 new cases occur each year. In a recent genome scan, we mapped a susceptibility gene for leprosy per se (all clinical forms) to chromosome region 6q25 (Mira et al,Nat Genet,2003). Within the linked 6q25 region, one gene presented appealing characteristics in the context of leprosy control. We constructed a dense map of this gene and identified 74 informative SNPs. These SNPs were used to perform a family-based association study in a sample of 208 Vietnamese trios with 2 parents and one affected offspring. Significant association ($p<0.005$) was found with several SNPs located in the promoter area of this gene. Fine linkage disequilibrium (LD) map of the promoter identified three main blocks of strong LD. Multivariate analyses showed that only two SNPs were needed to capture almost all association information. Based on these two SNPs, we identified a risk haplotype associated with a 5-fold increase in the risk of leprosy when compared to the protective haplotype ($p < 0.001$). In order to validate this result, a case-control study was carried out in an independent sample from Brazil including 708 cases and 394 controls matched on ethnicity. The very same pattern of results was observed with 10 out of the 15 selected SNPs being strongly associated with leprosy per se in this new sample (p-values ranging 0.03 to 10-4). Finally, we investigated expression of this gene by means of RT-PCR performed on RNA from different cell types. Interestingly, this gene was expressed in both macrophages and Schwann cells known to interact with M. leprae. Based on the predicted function of this gene, we postulate that a new physiopathological pathway implicated in the onset of leprosy has been identified.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 85 from 2003 ASHG Annual Meeting

Reversal of Purkinje cell pathology and motor dysfunction in conditional *SCA1* transgenic mice. *T. Zu*[1], *M.D. Kaytor*[1], *C.A. Vierra-Green*[1], *H.Y. Zoghbi*[2], *H.B. Clark*[1], *H.T. Orr*[1]. 1) Dept Lab Medicine and Pathology, Institute of Human Genetics, Univ of Minnesota, Minneapolis, MN; 2) Howard Hughes Medical Institute, Dept Molecular and Human Genetics, Baylor College of Medicine, Houston TX.

Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant neurodegenerative disorder characterized by ataxia, progressive motor dysfunction, and loss of cerebellar Purkinje cells. The current study was performed to determine the effects of stopping expression of mutant ataxin-1 on the phenotype in transgenic mice. Double transgenic mice were generated that express a tetracycline-regulated transactivator (tTA) driven by the Purkinje cell-specific promoter, *Pcp2*(*L7*); and an *SCA1* gene with 82 CAG repeats driven by a tetracycline responsive element (TRE). The mutant-SCA1 gene is expressed in the absence of doxycycline (dox), at levels equivalent to the SCA1[82Q] B05 line, and not in its presence. These mice had impaired rotarod performances and Purkinje cell pathology similar to age-matched B05 mice. Treatment with Dox between ages 6-12 weeks resulted in improved Purkinje cell morphology when compared to untreated tTA/TRE mice both at 6 and 12 weeks. Ataxia measured by cage behavior and rotarod also improved. Treatment with Dox between ages 12-16 weeks resulted in pathological and behavioral improvement when compared to untreated mice both at 12 and 16 weeks. After 8 weeks treatment with Dox, rotarod performances of these mice improved. Nuclear inclusions of ataxin-1 in Purkinje cells were absent after turning off the expression of mutant protein between ages 12-14 weeks. To better characterize the disappearance of nuclear inclusions after turning off the expression of mutant ataxin-1, the mice at 16 weeks of age were treated with Dox. 10 days after the treatment, the percentage of Purkinje cells with nuclear inclusion reduced from ~50 % to ~1.5%. In summary, our data show that the progression of SCA1 pathogenesis is dependent on the continuous expression of mutant ataxin-1. Mild pathology of Purkinje cells and motor dysfunction can be improved in a model of SCA1 if production of mutant ataxin-1 is halted. Moreover, nuclear inclusions can be cleared rapidly even at later stages of disease.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 86 from 2003 ASHG Annual Meeting

Functional studies of Af4 in the robotic mouse. *K.E. Davies[1], P.L. Oliver[1], E.L. Jones[1], A. Jeans[1], J. Clark[1], G. Doran[1], A. Potter[1], P.M. Nolan[2], L. Vizor[2], P. Glenister[2], S.D.M. Brown[2].* 1) Dept Human Anatomy & Genetics, Univ Oxford, Oxford, United Kingdom; 2) MRC Mammalian Genetics Unit, Harwell, Oxon, OX11 0RD, UK.

The robotic mouse is an autosomal dominant mutant that arose from the large-scale ENU mutagenesis programme based at Harwell, UK. It has a jerky, ataxic gait and develops adult-onset Purkinje cell loss in the cerebellum in a striking region-specific pattern as well as cataracts. Genetic and physical mapping of the disease locus led to the identification of an amino-acid substitution in a highly conserved region of Af4, a putative transcription factor known to play a role in leukaemogenesis.

To understand the role of Af4 in this new model of neurodegenerative disease, a number of approaches have been undertaken. Firstly, to model the effect of the mutation on the regulatory properties of Af4 and related proteins, in vitro transcriptional activation assays have been carried out in addition to DNA-binding experiments. A large microarray study of the robotic and wild-type cerebellum has also been completed and a number of genes have been isolated that are differentially regulated over a time-course reflecting the progression of cell loss that is observed in mutants. In addition, yeast-two hybrid studies have identified a number of binding partners in the brain that interact with both wild-type and robotic Af4, and experiments to determine the relative affinity of these proteins are being investigated. Finally, the robotic mutation occurs in a domain shared by Fmr2, a related gene involved in FRAXE mental retardation that is also expressed in Purkinje cells. Parallel studies with Fmr2 proteins are being carried out by over-expression of wild-type and robotic mutant proteins in neuronal cell lines.

Using this combinatory approach, we aim to explain the reasons behind the Purkinje cell death that occurs in the robotic mutant, and understand more about the function of the Af4 protein family within the CNS.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 87 from 2003 ASHG Annual Meeting

Maspardin is mutated in Mast syndrome, a form of hereditary spastic paraplegia associated with dementia. *M. Simpson*[1], *C. Proukakis*[1], *H. Cross*[2], *M.A. Patton*[1], *A.H. Crosby*[1]. 1) Dept Medical Genetics, St George's Hospital Medical School, London, UK; 2) Department of Ophthalmology, University of Arizona School of Medicine, 655 N. Alveron Way, Tuscon, USA.

Mast syndrome is an autosomal recessive complicated form of hereditary spastic paraplegia with dementia present at high frequency amongst the Old Order Amish. Using an extensive Amish pedigree, we have mapped the Mast syndrome locus (SPG21) to a small interval of chromosome 15q22.31 encompassing just three genes. Sequence analysis of the three transcripts revealed that all 14 affected cases were homozygous for a single base-pair insertion (601insA) in the acidic cluster protein of 33kDa (ACP33) gene. This frameshift results in the premature termination (fs201-212X213) of the encoded product, designated Maspardin, which has been previously shown to localise to intracellular endosomal/trans-Golgi transportation vesicles and may function in protein transport and sorting.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 88 from 2003 ASHG Annual Meeting

Down Syndrome Mental Retardation: DSCR2 identifies CNS axonal patterns and transgenic mice exhibit abnormal behavior. *J. Vesa[1], G. Barlow[1], M. Brennan[2], D. Patterson[2], G.E. Lyons[3], L.S. Crnic[4], J.R. Korenberg[1]*. 1) Med Genet, Cedars-Sinai Medical Center, UCLA, Los Angeles, CA; 2) Eleanor Roosevelt Institute, Denver, CO; 3) Dept. of Anatomy, Univ. of Wisconsin Medical School, Madison, WI; 4) Colorado Mental Retardation and Developmental Disabilities Research Center, Denver, CO.

Down Syndrome (DS) is a major cause of mental retardation caused by trisomy for chromosome 21. To elucidate the pathways underlying the abnormalities of the DS brain, we have characterized genes that are associated with cognitive defects of DS and studied their effects in transgenic mice. Of these, the DSCR2 gene encodes a novel protein with one potential N-glycosylation site and two predicted transmembrane domains. We now show that DSCR2 is expressed from E7.5, and exhibits differential patterns with tissue in situ hybridization and immunohistochemistry in the developing brain (E12-E16), heart and thymus. We further reveal striking distribution on subsets of mature CNS axons, including those of the neocortex, hippocampus and cerebellar Purkinje cells. In transiently transfected HEK-293 and COS-1 cells, DSCR2 is targeted to the cytoplasmic compartment, whereas in mouse primary cortical and retinal neurons the protein is found along axonal extensions. To further clarify the role of DSCR2 in CNS development and connectivity of the adult brain, we have generated a transgenic mouse model expressing human DSCR2, using a BAC containing the human gene and at least 20 kb of the putative endogenous promoter region. Two independent lines were shown to transmit the BAC transgene to progeny, and expression of the human DSCR2 mRNA was confirmed by RT-PCR. We now report that DSCR2 transgenic mice exhibit specific behavioral abnormalities, including anxiety on tests thought to involve higher-level inhibitory systems including the prefrontal cortex and hippocampus. In summary, we propose that DSCR2 is an exciting candidate contributing to the neurocognitive features of DS, whose over-expression in a transgenic mouse model causes behavioral abnormalities.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 89 from 2003 ASHG Annual Meeting

Synaptotagmin I and XI: parkin interaction, localization to Lewy bodies and cell death. *D.P. Huynh, D. Nguyen, S.M. Pulst.* Division of Neurology, Department of Medicine, Cedars-Sinai Medical Center-UCLA School of Medicine, Los Angeles, CA.

Inactivating mutations of the gene encoding parkin cause PARK2, an autosomal recessive form of Parkinson disease (PD). Parkin is an E3 ubiquitin ligase that regulates several proteins including -synuclein and synphilin that are also constituents of Lewy bodies. In a yeast two-hybrid screen, we identified synaptotagmin-I and XI as novel parkin binding proteins. Interaction was confirmed by co-immunoprecipitation of the endogenous proteins in substantia nigra extracts and by demonstrating that parkin ubiquitinates both synaptotagmins. Truncated parkins or parkins with disease-causing amino acid substitutions had reduced or absent binding and ubiquitination. In sporadic PD brain, sytXI was found in the core of the Lewy bodies. Overexpression of either sytI or sytXI was highly toxic to cultured cells and caused cell death in 25-50% of human embryonic kidney 293 and PC12 cells within 24 hrs. Cell death was markedly inhibited by co-expression of parkin. In conclusions, as both sytI and sytXI are important for vesicle formation and docking, the regulation of these proteins suggests a role for parkin in presynaptic neurotransmission. Altered synaptotagmin regulation as a consequence of parkin loss may explain the deficits in dopaminergic function seen in patients with parkin mutations. As unregulated expression of synaptotagmin results in cell death, these findings support the increasing body of data that proper regulation and trafficking of synaptic proteins are important for neuronal survival. *Supported by R01 NS33123, the National Ataxia Foundation, and the Carmen and Louis Warschaw endowment for neurolgoy*.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 90 from 2003 ASHG Annual Meeting

Functional analysis of yeast and human NADH kinase in *Saccharomyces cerevisiae*: implications for mitochondrial genome stability and neurodegenerative disease. *K.V. Shianna[1], H. Hullinger[1], M.K. Strand[2], D.A. Marchuk[1].* 1) Dept. Mol. Genet. and Microbiol., Duke Univ Medical Ctr, Durham, NC; 2) Lab. Mol. Genet., NIEHS, RTP, NC.

An emerging pathological theme for many neurodegenerative disorders, as well as the process of aging itself, is an increase in the number of mutations in the mitochondrial genome. The molecular basis for this increase is just beginning to be elucidated in mammals, but is more genetically tractable in smaller eukaryotic systems. In the yeast *Saccharomyces cerevisiae*, the insertional inactivation of the *POS5* gene (mitochondrial NADH kinase) results in a significant increase in the mitochondrial mutation rate as measured by petite frequency and other established methods. We sought to exploit this system to further elucidate the role of NADH kinase in mitochondrial genome stability. When the *pos5* knockout (KO) is combined with the cytoplasmic NADH kinase KO (*UTR1* gene), lethality results. Exploiting this easily scoreable phenotype, we showed that the human NADH kinase cDNA complements the lethality, suggesting that the human homologue of this gene may also play a role in mitochondrial genome stability. The *pos5* KO also results in an increase in the level of iron in the mitochondria, similar to the frataxin KO (model for Freidreich's Ataxia) in yeast. In order to further elucidate the biochemical pathways involved in mitochondrial stability, we have performed microarray analysis on the *pos5*, *utr1*, and YEL041w (a close homolog to *UTR1*) knockouts. In the *pos5* KO many of the key iron transport genes have an increased expression, and show similarity to the frataxin KO expression pattern, supporting a role of iron homeostasis in mitochondrial stability. In summary, using a yeast model system, we have shown that NADH kinase is a critical enzyme for mitochondrial genome stability, and that its effects may be due to increased oxidative stress and altered iron homeostasis. Since human NADH kinase complements the yeast phenotype, this gene is a compelling biological candidate gene for mutation in many neurodegenerative diseases that exhibit increased mitochondrial mutation rates.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 91 from 2003 ASHG Annual Meeting

A Mouse Model of Cytochrome c Oxidase Deficiency in Muscle. *F. Diaz, C.T. Moraes.* Neurology, University of Miami, Miami, FL.

Cytochrome c oxidase (COX) is the terminal complex of the electron transport chain and has been associated with multiple neuromuscular disorders and neurodegenerative diseases. COX biosynthesis depends on a large number of integral subunits as well as on assembly factors. Studies in *S. cerevisiae* showed that incorporation of heme a in the catalytic site of the complex requires several enzymes that modify the protoheme backbone, among which is the product of COX10, a protoheme:heme O farnesyl trasferase. The COX10 gene is extremely conserved in evolution and the human counterpart has been found to be mutated in a family with a leukodystrophy and tubulopathy. We created a conditional knockout mouse with a COX10 exon 6 flanked by loxP sites. Crossing these mice with a mouse expressing the Cre-recombinase in skeletal muscle (under the myosin light chain promoter), produced mice that were smaller and showed progressive loss of skeletal muscle mass. These mice had extremely reduced endurance in a treadmill and sweated profusely when forced to exercise. Histological analyses of muscle showed severe, but patchy COX deficiency, results that were confirmed by biochemical assays. The effect of COX10 depletion in COX assembly is currently under investigation. To our knowledge, this is the first animal model of an isolated respiratory chain deficiency, which should be instrumental in helping us understand the pathophysiology of these diseases as well as in developing treatments.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 92 from 2003 ASHG Annual Meeting

An endogenous retroviral LTR acts as a tissue-specific promoter for the human 1,3-galactosyltransferase 5 gene.

C.A. Dunn[1,3], P. Medstrand[2], D.L. Mager[1,3]. 1) Terry Fox Laboratory, BC Cancer Agency, Vancouver, British Columbia, Canada; 2) Dept. of Cell & Molecular Biology, Lund University, Lund, Sweden; 3) Dept. of Medical Genetics, Univ. of British Columbia.

Remnants of endogenous retroviruses (ERVs) and related solitary long terminal repeats (LTRs) make up 8% of the human genome. These LTRs often retain the ability to regulate transcription and are known to serve as tissue-specific promoters for several human genes, usually as one of multiple alternative promoters. In a screen to detect examples of this phenomenon, we identified 1,3-galactosyltransferase 5 (3Gal-T5) as a candidate gene regulated by an LTR. 3Gal-T5 is thought to be responsible for the synthesis of type 1 Lewis antigens in colorectal and pancreatic cancer cell lines. We determined that the first exon and promoter of the human gene are derived from an LTR of the ERV-L family. The LTR was found to be present in Old World, but not New World monkeys, dating its insertion into the genome at 25-30 million years ago. Using RT-PCR and 5'-RACE methods, we determined that the LTR is one of at least two alternative promoters for the 3Gal-T5 gene. Real-time PCR was carried out to quantify 3Gal-T5 expression and the contribution of the LTR to total transcription in a range of normal human tissues. The LTR promoter was most active in tissues of the gastrointestinal tract and the mammary gland and, indeed, represents the dominant promoter in the colon. Intriguingly, although the ERV-L LTR is not present in the mouse, the murine homologue of 3Gal-T5 was expressed in the tissues where the human LTR is most active, suggesting that different mechanisms have evolved to give similar expression patterns in the two species. Functional analysis of the human LTR promoter was carried out in colorectal cancer cell lines. Putative binding sites for the tissue specific transcription factor HNF-1alpha, as well as the basal factors NF-Y and Sp1, were identified and found to be important for LTR promoter activity. These studies demonstrate that an ancient LTR plays an important role in tissue-specific regulation of the 3Gal-T5 gene in humans.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 93 from 2003 ASHG Annual Meeting

X inactivation-specific methylation of LINE-1 elements by DNMT3B: implications for the Lyon repeat hypothesis of X inactivation spreading. *R.S. Hansen.* Division of Medical Genetics, Box 357720, University of Washington, Seattle, WA 98195.

Lyon has proposed that long interspersed nuclear element 1 (LINE-1 or L1) repeats may be mediators for the spread of X chromosome inactivation. Cells from ICF patients who are deficient in one of the DNA methyltransferases, DNMT3B, provide an opportunity to explore and refine this hypothesis. Southern blot and bisulfite methylation analyses indicate that in normal somatic cells, X-linked L1s are hypermethylated on both the active and inactive X chromosomes. In contrast, ICF syndrome cells with *DNMT3B* mutations have L1s that are hypomethylated on the inactive X, but not on the active X or autosomes. The DNMT3B methyltransferase, therefore, is required for methylation of L1 CpG islands on the inactive X, whereas methylation of the corresponding L1 loci on the active X, as well as most autosomal L1s, is accomplished by another DNA methyltransferase. This unique phenomenon of identical allelic modifications by different enzymes within the same cell has not been previously observed. Apart from CpG island methylation, the ICF inactive X is basically normal in that it forms a Barr body, is associated with XIST RNA, mostly replicates late, and its X-inactivated genes are mostly silent. Because the unmethylated state of the ICF inactive X L1s probably reflects their methylation status at the time of X inactivation, these data suggest that unmethylated L1 elements, but not methylated L1s, may have a role in the spreading of X chromosome inactivation.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

A comprehensive X inactivation profile of the human X chromosome. *L. Carrel[1,2], G. Nickel[1], K. Trevarthen[1], J. Dunn[1], H.F. Willard[1,3].* 1) Case Western Reserve Univ, Cleveland, OH; 2) Penn State Univ, Hershey, PA; 3) Duke Univ, Durham, NC.

In females, most genes on the inactive X are silenced as a result of X inactivation. However, an increasing number of genes are known to escape inactivation and are expressed from both the active (Xa) and inactive (Xi) X. We present a profile of 670 genes of the estimated total of ~1000 genes on the human X, representing essentially all genes that are expressed in readily assayable tissues. Of 950 transcripts initially evaluated, 670 showed robust expression in human fibroblasts. Xi expression was then tested in 9 mouse/human hybrids containing a human Xi, but no Xa. Pseudoautosomal genes (n=11) escaped inactivation as expected. Of the remainder, 480 (73%) were subject to inactivation, 106 (16%) escaped inactivation, and 11% showed variable expression, being expressed in about half of the Xi hybrids. The proportion of genes escaping inactivation differed dramatically in different regions of the X. Only 7.5% of 454 genes on Xq and the ancestral portion of proximal Xp escaped inactivation. In contrast, 33% of 205 genes in the more recently added portion of Xp escape inactivation, strongly suggesting an inactivation mechanism based on the genome sequence of the ancestral X. To validate these data, Xi expression of ~100 genes was also evaluated in human cells by assaying expressed SNPs in a panel of 48 fibroblast lines with complete non-random inactivation. The same general profile emerged; the frequency of genes escaping inactivation was 5-fold higher on Xp than on Xq. In addition, 5-10% of genes were subject to inactivation in some cell lines, but escaped inactivation in others, confirming the Xi hybrid findings. This suggests a previously unsuspected degree of heterogeneity among females. These data have implications for considering phenotypes associated with abnormal Xs, as dosage imbalance for the ancestral portion of the X would be expected to have much less severe consequences than imbalance involving most of Xp. In addition, this comprehensive X inactivation profile provides insight into the genomic, epigenetic and evolutionary organization of the X.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 95 from 2003 ASHG Annual Meeting

Forced myogenesis of fibroblasts can unmask mitochondrial respiratory chain (RC) defects. *J. Christodoulou[1, 2], J. Minchenko[1, 2], D.R. Thorburn[3], S. Cooper[4].* 1) Metabolic Research Unit, Childrens Hospital at Westmead, Sydney; 2) School of Paediatrics & Child Health, University of Sydney; 3) Murdoch Childrens Research Institute, Melbourne; 4) Neurogenetics Research Unit, Childrens Hospital at Westmead, Sydney, Australia.

In up to half of patients with proven RC defects based on tissue enzyme studies, the functional defect is not replicated in cultured fibroblasts (fbs). Myo-D, a master myogenic regulatory factor can induce myogenesis in non-muscle cells for studies of muscle proteins. We have used an adenoviral Myo-D vector to induce cultured fbs from patients with RC defects to differentiate into myotubes in order to examine whether the RC defect might be unmasked. Fbs were used from a normal pediatric control (fb complex IV [COX]/citrate synthase [CS] 40.67 +/- 3.3), a patient (MM) known to be a compound heterozygote for two SURF1 mutations (muscle COX/CS 22% of controls; fb COX/CS 10% of control), and a child (CG) with infantile onset transfusion-dependent sideroblastic anemia, lactic acidosis, and a slowly progressive myopathy, but with preserved intellect (muscle Complex I/CS 9% of controls; muscle COX/CS 21% of controls; fb COX/CS 61%). Fbs grown to 90-95% confluence were transduced with a Myo-D adenovirus containing murine myo-D cDNA (pAdmyoD). These cells were then differentiated in supplemented DMEM/HF12 media. After 7 days of differentiation, samples were harvested for analysis, including western blots and biochemical assays (COX, CS, protein). Control cells differentiated to myotubes did not change significantly (myotube COX/CS 91% of control fbs, $p = 0.075$), nor did myotubes from MM (88% of patient fbs, $p = 0.315$). In contrast, myotubes from CG showed a marked fall in COX/CS in myotubes (44% of patient fbs, $p = 0.004$; 30% of control myotubes). We conclude that in some cases where fbs do no show a functional RC defect seen in other tissues, forced myogenesis may unmask the defect. This could potentially pave the way for prenatal diagnosis using cultured chorionic villus cells where this was not previously possible and facilitate gene identification by allowing somatic cell genetic studies.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 96 from 2003 ASHG Annual Meeting

Diagnostic strategy for mucopolysaccharidoses and mucolipidosis. *S. Tomatsu[1], K. Okamura[4], H. Maeda[4], T. Taketani[3], S. Velez-Castrillon[1], M. Gutierrez[1], K. Orii[1], K. Isogai[2], W. Sly[1], T. Orii[2], S. Yamaguchi[3]*. 1) Dept Biochem & Molecular Biol, St Louis Univ Sch Medicine, St Louis, MO; 2) Department of Pediatrics, Gifu University School of Medicine; 3) Department of Pediatrics, Shimane Medical University; 4) Seikagaku Corporation, Tokyo Japan.

Background: Mucopolysaccharidoses (MPS) and mucolipidosis (ML) are progressive lysosomal storage diseases. MPS is characterized by accumulation of glycosaminoglycans (GAGs) and ML, by accumulation of GAGs and sphingolipids. To prevent progression of the disease, early diagnosis and treatment are required. We aim to assess a rapid diagnostic test for MPS and ML. Methods: One hundred thirty-nine blood and 232 urine specimens from MPS and ML patients were tested using the ELISA-Sandwich method with monoclonal antibody specific to keratan sulfate (KS). Findings: We found that each type of MPS not only involves excretion of the specific GAG species previously understood, but also produces excretion of above normal amounts of KS. Blood and urine KS concentrations varied with age. The lowest KS concentration was observed in the cord blood of control newborns (mean value: 40 ng/ml) while three newborn patients had 89.1 (MPS VII), 95.1 (MPS I), and 145 (ML II) ng/ml, respectively. Blood KS concentrations in patients under age 1 (mean, 206 ng/ml) were between 89 and 427 ng/ml, while those in the control population were between 19 and 110 ng/ml (mean, 69 ng/ml, $p<0.0001$). The highest concentration was between age 5 and 10 (mean, 234 ng/ml for controls vs. 700 ng/ml for patients; $p<0.0001$). The urine KS in patients under age 1 was significantly higher than in controls (0.72 vs. 0.22 mg/g creatinine; $p<0.0001$). Interpretation: These findings suggest that measurement of a single GAG (KS) may provide a screening test to enable detection of all types of MPS and ML.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 97 from 2003 ASHG Annual Meeting

Characterization of indeterminate Tay-Sachs screening results. *E.L. Schlenker[1], E.A. Sugarman[3], F. Myrick[2], A.E. Donnenfeld[1], S.M. Puck[2].* 1) Genzyme Genetics, Philadelphia, PA; 2) Genzyme Genetics, Santa Fe, NM; 3) Genzyme Genetics, Westborough, MA.

Purpose: To determine the incidence of Tay-Sachs mutations among individuals with indeterminate hexosaminidase A results. Materials and Methods: Between October 2001and April 2003, 15,049 samples were tested for Tay-Sachs carrier status by enzyme analysis on leukocytes. The hexosaminidase A (HexA) levels were determined by the heat inactivation technique. In our laboratory, the carrier range is 20-49%, the noncarrier range is ≥55% and the indeterminate range is >49% and <55%. Of the total, 566 (3.8%) had HexA levels in the indeterminate range. DNA analysis was performed on 422 (74.6%). The 8 alleles analyzed included the common Ashkenazi Jewish mutations (1421+1G>C, +TATC1278 and the late-onset G269S); common French Canadian mutations (7.6 kb deletion and IVS7+1G>A); a common non-Jewish mutation (IVS9+1G>A); and two pseudodeficiency alleles (R247W, R249W). Results: Disease causing mutations were identified in 46 of the 422 patients (10.9%). Of these, 38 patients were carriers of infantile Tay-Sachs disease mutations and 8 were carriers of the late-onset disease mutation. An additional 11 patients had pseudodeficiency alleles (2.6%), providing a likely explanation for the biochemical results but not conferring an increased risk for offspring affected with Tay-Sachs disease. The mean HexA level in the DNA positive samples was 51.48% and in the DNA negative group was 53.01%(p<.0001). Ashkenazi Jewish patients with an indeterminate enzyme result had a 16.9% chance to have a mutation identified, compared to 10.6% in patients of other ethnic backgrounds (p=.0591). A small number of patients (33) with indeterminate HexA results and negative DNA results had repeat biochemical testing. Of these, 25 were found to be noncarriers on repeat assay and 8 tested in the indeterminate range. Conclusions: Our findings show that true mutations or pseudodeficiency alleles will be present in 13.5% of patients with indeterminate Tay-Sachs enzyme results.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 98 from 2003 ASHG Annual Meeting

Can drugs enhance mRNA splicing efficiency in human genetic disease? *S. Slaugenhaupt[1,2], J. Mull[1], M. Leyne[1], S. Gill[1], M. Hims[1,2], M. Cuajungco[1,2], R. Reed[2], J. Gusella[1,2]*. 1) Massachusetts General Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA.

It has been estimated that as many as 15% of all mutations that cause genetic disease result from defective splicing of mRNA, and splicing mutations that result in exon skipping have been reported for a large number of disorders. These mutations are often associated with a milder disease phenotype due to low levels of normal transcript production. Thus, direct targeting of the splicing pathway to enhance normal transcript levels presents an exciting therapeutic option. Recently, we reported that a non-coding mutation in the gene IKBKAP is involved in all known cases of familial dysautonomia (FD, HSAN III). The major FD mutation is a single-base change in the donor splice site of intron 20 (IVS20+6 T-C) that results in an apparent decrease in splicing efficiency and variable skipping of exon 20. Interestingly, despite the fact that FD is a recessive disease, homozygous mutant cells are capable of expressing normal mRNA and protein. Using both densitometry and QPCR, we have shown that the relative efficiency of accurate splicing varies dramatically between tissues, with nervous system tissues displaying the lowest amount of normal IKBKAP mRNA. In an effort to develop effective therapies for FD patients, we have used a cellular splicing assay to perform a screen for drugs that alter splicing efficiency as part of the NINDS sponsored Neurodegeneration Drug Screening Consortium. We have now completed our second-round triplicate screen and have identified 5 compounds that significantly increase the wild-type to mutant transcript ratio. For the most active compound we have shown that the effect on splicing is dose dependent and consistent regardless of the drug source. Given these exciting results, we are testing these compounds for activity in other disorders that are due to IVS+5 and IVS+6 mutations and initiating studies to determine the mechanism of action of the compounds. Our studies suggest that targeting defective splicing rather than individual gene function may uncover potential treatments that will be applicable to a vast array of human genetic disorders.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Phenotypic Correction of Canine Hemophilia A with a Helper-dependent Adenoviral Vector Encoding Canine FVIII. *W.M. McCormack[1], K. Ubhayakar[1], V. Mane[1], M. Guenther[1], T. Nichols[3], P. Ng[1], D. Palmer[1], A. Beaudet[1], B. Lee[1,2]*. 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX; 3) Pathology & Laboratory Medicine, University of North Carolina, Chapel Hill, NC.

Hemophilia A is a bleeding disorder due to a deficiency of coagulation factor VIII (FVIII). Gene therapy has been proposed as an alternative treatment to exogenous factor replacement. Expression of as little as 1-5% normal FVIII can significantly correct the phenotype greatly improving the quality of life for these patients. Adenoviral vectors efficiently transduce liver, the endogenous source of Factor VIII. However, earlier generation adenoviral vectors displayed limited utility due to short duration of transgene expression and toxicity secondary to expression of adenoviral proteins. Helper-dependent adenoviral vectors (HDV) are devoid of all viral coding regions greatly reducing toxicity and extending transgene expression. In this study, two severe hemophilia A dogs were treated via peripheral vein injection with a HDV encoding canine FVIII driven by a liver-restricted promoter. A low dose animal received 1 x 10^{12} viral particles/kilogram (vp/kg) and a higher dose animal received 3 x 10^{12} vp/kg. Whole blood clotting time (WBCT) in the low dose animal dropped from a baseline of 44 to 16 minutes by day 6 (normal=8-12 minutes) and slowly increased to 25 minutes over the next 3 months. A peak of 1.4% normal FVIII by bioactivity assay was reached at 17 days. There was no elevation of hepatic transaminases and platelets remained within the normal range. In the high dose, animal WBCT dropped from a baseline of 40 to 8.5 minutes after the first week and has remained in the normal range (8-12 minutes) for over 2 months. FVIII level reached a peak of 70-80% normal by day 14 and has subsequently declined. There was a slight elevation of hepatic transaminases that returned to baseline by 48 hours after injection. Platelets dropped to 100,000/mm^3 by day 2 and returned to the normal range by day 7. These preclinical data demonstrate prolonged correction of hemophilia A in a large animal model with minimal toxicity.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 100 from 2003 ASHG Annual Meeting

Gene Therapy of Hemophilia A: Total correction of hemophilia A mice and partial correction of hemophilia A dogs with canine FVIII cDNA using the AAV8 serotype. *R. Sarkar[1], R. Tetreault[1], G.P. Gao[1], L. Wang[1], P. Bell[1], R. Chandler[1], D. Bellinger[2], T.C. Nichols[2], J.M. Wilson[1], H.H. Kazazian[1].* 1) Univ Pennsylvania, School of Medicine, Philadelphia, PA; 2) Univ North Carolina, Chapel Hill, NC.

AAV2 is considered safe with prolonged transgene expression. However, its application is limited by packaging constraints and poor expression levels. Here we had three objectives. (1) To compare AAV serotypes 2, 5, 7 and 8, expressing B domain deleted canine FVIII cDNA, delivered intraportally to hemophilia A mice either as two separate vectors or as a single vector. Doses used were $1x10^{10}$, $3x10^{10}$, and $1x10^{11}$ gc/vector/mouse. (2) To assess efficacy of two separate vectors versus a single FVIII vector in hemophilia A mice, and (3) To evaluate the efficacy of these vectors in hemophilia A dogs. Canine FVIII cDNA was obtained by RT-PCR of normal dog liver RNA. Primers were designed specifically to eliminate the B domain while amplifying the heavy and light chains of canine FVIII cDNA. The two chain PCR fragments were brought into frame to create a 4.5 Kb single chain version of the construct. All three constructs were driven by a liver-specific promoter. The serotype comparison demonstrated the performance of AAV 8 to be superior to all other serotypes. Although near normal levels of FVIII activity were seen with AAV 7 at the high dose, barely detectable levels were obtained with AAVs 5 and 2. The results clearly demonstrate a t least 100% correction in mice, beyond 12 months with the AAV 8 serotype, delivered either as two separate vectors or as a single vector. Two hemophilia A dogs were treated. One with a vector dose of $1.25x10^{13}$ gc/vector/Kg maintained 6-8%; of normal canine FVIII activity for 9 months. The second dog injected with twice as much vector had 2.5% FVIII activity at 7 months. These levels are consistent with conversion from severe to mild to moderate forms of hemophilia A. These results suggest that AAV 8 is useful in liver-directed gene transfer for FVIII deficiency.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 101 from 2003 ASHG Annual Meeting

Functional renal tubular epithelium can emerge from adult bone marrow. *P.K. Held*[1], *M. Al-Dhalimy*[1], *M. Finegold*[2], *M. Gibson*[1], *Y. Akkari*[1], *M. Grompe*[1]. 1) Molecular and Medical Genetics, Oregon Health & Sciences University Portland, Oregon, United States; 2) Department of PathologyBaylor College of MedicineHouston, Texas, United States.

Hematopoietic stem cell (HSC) transplantation results in liver repopulation in a mouse model of Hereditary Tyrosinemia Type I (HTI). HTI mice are deficient in fumarylacetoacetate hydrolase (FAH), which is normally expressed in both hepatocytes and renal proximal tubules, and exhibit both liver and kidney damage. Therefore, we wished to test whether hematopoietic stem cells could also repopulate the damaged renal tubule epithelial cells in order to correct the renal damage in this mouse. Bone marrow transplants were performed in lethally irradiated FAH-/- mice and the positive selection for wild-type cells was initiated by removing mice from the protective drug, NTBC. After six months of selection, however, no donor derived renal tubules were seen in the kidney of the recipient mice. Because the renal damage in liver repopulated FAH mutant mice was minimal, we next developed a model of chronic renal regeneration. Mice which are FAH mutant but heterozygous for homogentisic acid dioxygenase (HGD), an enzyme up stream in the tyrosine degradation pathway, undergo spontaneous loss of heterozygosity in hepatocytes, resulting in repopulation of the liver by HGD deficient hepatocytes. This causes elevated levels of homogentisic acid (HGA) and chronic renal tubular injury. FAH-/-HGD+/- mice were lethally irradiated and transplanted with bone marrow cells from a ROSA26 donor. Seven months after transplantation two surviving mice were analyzed. Both animal showed normal renal morphology. In addition, they showed extensive (>50%) replacement of their renal proximal tubular cells by donor derived cells. This result shows that adult bone marrow contains cells capable of repopulating damaged renal epithelium. In addition, the FAH-/-HGD+/- mouse is the first animal model for positive selection of transplanted renal progenitor cells. We are currently investigating whether the kidney repopulating bone marrow cells display the same cells surface marker as a HSC.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 102 from 2003 ASHG Annual Meeting

Association of Ectonucleotide Pyrophosphatase/Phosphodiesterase 1 (ENPP1) Polymorphisms with Insulin Resistance in Nondiabetic Mexican Americans. *D.K. Richardson, R. Arya, J. Schneider, L.M. Rodriguez, P.S. Streng, R. Duggirala, M.P. Stern, J. Blangero, L. Almasy, L.J. Mandarino, R.A. DeFronzo, C.P. Jenkinson.* San Antonio, TX.

ENPP1 has been implicated in type 2 diabetes mellitus and obesity. Increased expression of ENPP1 and/or expression of variant forms of ENPP1 attenuate insulin signaling by binding to the insulin receptor. The ENPP1 gene is located at chromosome 6q23, a region that has been strongly linked to diabesity phenotypes in Mexican Americans (LOD=5.4 for bivariate phenotype, fasting specific insulin [FSI]/leptin). This region has been linked to related phenotypes in multiple studies and appears to harbor a pleiotropic diabesity gene. We genotyped SNPs within a 90kb interval encompassing the gene in 429 subjects from the San Antonio Family Diabetes Study (SAFADS), using allelic discrimination PCR. We tested SNPs for association with FSI and BMI using a variance components approach. The FSI analyses used 293 non-diabetic subjects (mean FSI = 127.9 pM) and BMI analyses were performed on all 429 subjects (mean BMI = 30.0 kg/m2). Of 24 SNPs examined, two (SNPs 9 and 24, both located in intron 1) were associated with BMI (p = 0.02 and 0.05, respectively) and one of these (SNP 24) was marginally associated with FSI (p = 0.06). Key functional SNPs (K121Q and mRNA-stabilizing SNPs in the 3'UTR) also were genotyped in a large subset of subjects from the Veterans Administration Genetic Epidemiology Study (VAGES) (N = 680). Significant associations were found for traits such as: Waist to Hip Ratio (K121Q, p = 0.002); 2h plasma glucose (OGTT) (3'UTR, p = 0.01); mean 2h plasma insulin (OGTT) (3'UTR, p = 0.01) and insulin secretion index (I/G during OGTT) (3'UTR, p = 0.02). These results are in agreement with previous findings and establish ENPP1 as a diabesity susceptibility gene. We are enthused by these findings and further analyses are planned with the remaining phenotypes and SNPs.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 103 from 2003 ASHG Annual Meeting

Two new loci linked to altered lipoprotein particle size in obese individuals. *M. Olivier[1], L.J. Martin[2], J. Blangero[3], A.G. Comuzzie[3], D.L. Rainwater[3], G.E. Sonnenberg[1], A.H. Kissebah[1].* 1) Medical College of Wisconsin, Milwaukee, WI; 2) Childrens Hospital Medical Center, Cincinnati, OH; 3) Southwest Foundation for Biomedical Research, San Antonio, TX.

One of the hallmarks of the metabolic syndrome is an altered lipid and lipoprotein profile, including elevated plasma triglyceride levels, decreased HDL cholesterol levels, and predominance of small dense LDL and HDL particles. These changes have been recognized as highly predictive of several morbidities including coronary artery disease. As part of our investigation into the genetic basis of these lipid profile abnormalities, we measured LDL peak particle diameter (LDLp) and HDL median diameter (HDLm) using nondenaturing gradient gel electrophoresis in 532 adult individuals of predominantly northern European ancestry distributed across 85 families from our study cohort. All individuals were genotyped for 387 markers yielding an average map density of approximately 10cM. Linkage analysis was conducted using a variance component approach implemented in the program package SOLAR. We adjusted HDLm for sex as covariate, and LDLp was adjusted for sex, triglycerides, and waist-hip ratio. Using quantitative genetic analysis, we detected an additive genetic heritability of 53% and 36% for HDLm and LDLp, respectively. The maximum LOD score detected for HDLm in a multipoint linkage scan was 3.15 ($p=7.0x10^{-5}$) on chromosome 12. The maximum LOD score detected for LDLp in a multipoint linkage scan was 3.06 ($p=8.7x10^{-5}$) on chromosome 8. In bivariate analyses, LDLp was moderately correlated to plasma triglyceride, adiponectin, and insulin levels, while HDLm showed correlation with anthropomorphic measures such as waist-hip ratio. This analysis identifies two new loci influencing the complex lipid and lipoprotein profile of patients with the metabolic syndrome. These loci are independent from other loci linked to plasma lipid levels in our cohort as well as other previously reported loci linked to plasma lipid and lipoprotein levels. Furthermore, bivariate analysis clearly illustrates the intricate interactions of obesity, altered lipid profile, and insulin resistance in the metabolic syndrome.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Glutathione S-Transferase, Omega-1 (GSTO1) modifies age at onset of Alzheimer Disease and Parkinson Disease. *J.M. Vance[1], Y. Li[1], A.S. Oliveira[1], M.A. Hauser[1], E.R. Martin[1], W.K. Scott[1], J.E. Stenger[1], C. Scherzer[2], S.R. Gullans[2], G.W. Small[3], A.D. Roses[4], A.M. Saunders[4], D.E. Schmechel[1], K.A. Welsh-Bohmer[1], C. Hulette[1], J.L. Haines[5], J.R. Gilbert[1], M.A. Pericak-Vance[1], Parkinson Disease Genetics Collaboration.* 1) Duke Univ Medical Ctr, Durham, NC; 2) Harvard Univ. Med. School, Boston, MA; 3) Univ. of California, Los Angeles, CA; 4) GlaxoSmithKline Research Directorate, RTP, NC; 5) Vanderbilt Univ. Med. Ctr., Nashville, TN.

We previously reported genetic linkage of loci controlling age-at-onset in Alzheimer (AD) and Parkinson disease (PD) to a 15 cM region on chromosome 10q. Given the large number of genes in this initial starting region, we applied our process of genomic convergence to prioritize and reduce the number of candidate genes for further analysis. As our second convergence factor we performed gene expression studies on hippocampus obtained from AD patients and controls. Analysis revealed that four of the genes (Stearoyl-CoA desaturase (SCD); NADH-ubiquinone oxidoreductase 1 beta complex 8 (NDUFB8); protease, serine 11 (PRSS11) and glutathione S-transferase, omega-1 (GSTO1)) were significantly different in their expression between AD and controls, and also mapped to the 10q age-at-onset linkage region. Using 1773 AD and 635 PD patients and their relatives, allelic association studies in AD and PD demonstrated no association for age-at-onset with three of the candidates, but a significant association with AD ($p=0.001$) as well as the smaller PD dataset ($p=.03$) was found for GSTO1 and a second transcribed member of the GST omega class, GSTO2, located near GSTO1. Association studies for GSTO1 and GSTO2 in the combined AD and PD data set was also highly significant ($p<0.004$). The function of GSTO1 and GSTO2 is not well understood, but believed to involve different substrates than most members of the glutathione S-transferase protein family. GSTO1 has recently been shown to be involved in the post-translational modification of the inflammatory cytokine Interleukin-1. This is provocative given reports of the possible role of inflammation in these two neurodegenerative disorders.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 105 from 2003 ASHG Annual Meeting

Systematic linkage disequilibrium analysis of SLC12A8 at PSORS5 suggests a role in susceptibility to psoriasis arthritis rather than psoriasis vulgaris. *A. Reis*[1], *U. Huffmeier*[1], *B. Bohm*[2], *J. Lehmann*[3], *F. Schurmeier-Horst*[4], *J. Wendler*[5], *H. Traupe*[4], *H. Burkhardt*[2]. 1) Inst. of Human Genetics, Univ. Erlangen-Nuremberg, Germany; 2) Dept. of Internal Medicine III (Rheumatology), Univ. Erlangen-Nuremberg; 3) Psoriasis rehabilitation clinic, Bad-Bentheim; 4) Dept. of Dermatology, Univ. Munster; 5) Private practice of Rheumatology, Erlangen.

The gene for the soluble co-transporter SLC12A8 has recently been proposed as a candidate gene for psoriasis susceptibility (PSORS5) on chromosome 3q based on association of various intronic SNPs in a Swedish psoriasis cohort. We attempted to replicate this finding in a group of 210 trios with psoriasis originating from Germany but failed to identify significant association using TDT statistics. Since in a previous study linkage to this locus was stronger when families were stratified according to joint complaints we hypothesized SLC12A8 might be a susceptibility locus for psoriasis arthritis rather than psoriasis vulgaris. We therefore recruited an independent cohort of 199 psoriasis arthritis patients ascertained through a rheumatology clinic and investigated previously reported SNPs for association in a case control design. We detected significant association to one intronic SNP B1551S3 (^{2} =23.36; p<0.0001) supporting this hypothesis. Next we determined the haplotype structure of SLC12A8 through systematic linkage disequilibrium analyses. We assayed a total of 18 haplotype tag SNPs which we grouped into eight LD-blocks of 10-30 kb size each. We tested these haplotypes for association and again failed to detect association in the psoriasis cohort. In contrast, we detected strong association in the psoriasis arthritis cohort to the haplotype encompassing B1551S3 (^{2} =53.64; p<<0.0001) with an OR =3.92 (2.62-5.88). The other haplotypes showed no association. Our findings indicate that SLC12A8 is rather a susceptibility locus for psoriasis arthritis than for psoriasis vulgaris and confirm the different nature of these two conditions. We conclude that a variant within the associated haplotype is involved in the aetiology of this disorder and further genetic as well as functional analyses are required to identify its exact nature.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 106 from 2003 ASHG Annual Meeting

Linkage and association to the NOS2A locus on ch.17q11 in multiple sclerosis (MS). *L.F. Barcellos[1], A.B. Begovich[2], S.J. Caillier[1], S. Schmidt[3], D. Brassat[1], B.A.C. Cree[1], L. Steiner[2], M.A. Pericak-Vance[3], J.L. Haines[4], H.A. Erlich[2], R. Reynolds[2], S.L. Hauser[1], J.R. Oksenberg[1], MS Genetics Group.* 1) Dept. of Neurology, UC San Francisco; 2) Roche Molecular Systems, Inc., Alameda, CA; 3) Duke University Med Center, Durham, NC; 4) Vanderbilit University, Nashville, TN.

A large body of research supports a complex etiology in MS. We used a highly efficient multilocus genotyping assay to study SNPs within 34 genes from inflammatory pathways in a well-characterized MS dataset. Significant evidence for transmission distortion using the pedigree disequilibrium test was present for NOS2A (exon 10 C/T, D346D) on ch.17q11 in 427 families (p=0.009); this association was more prominent in families carrying the HLA-DR2 (DRB1*1501-DQB1*0602) haplotype (p=0.0006). These results were replicated in a second dataset comprised of 442 African-American patients and 293 controls (p=0.02); in both cases, an increased frequency of the less common (T) allele was present in patients. Three additional NOS2A polymorphisms including two promoter variants, (CCTTT)n and (TAAA)n, and a SNP within exon 16 (C/T, S569L), and three flanking markers (D17S1800, D17S798, D17S749) were selected for further study. In DR2 positive families, significant results were observed for the exon 16 SNP (p=0.03) and D17S798 (p=0.03). Haplotype analyses were performed for NOS2A SNPs using TRANSMIT. Significant results were observed for haplotypes comprised of exon 10 and 16 alleles(p=0.03 and p=0.0024, in all, and DR2 positive families, respectively, global test) and specifically, for the exon 10-allele2(T)-exon 16-allele2(T) haplotype (p=0.0009). Although no associations were observed for any ch.17q11 loci in a third dataset of 186 multicase families, evidence for linkage was present for the (CCTTT)n promoter polymorphism (HLOD=2.4), and was also restricted to DR2 positive families (HLOD=2.7). For the first time, our results provide strong evidence for linkage and association to a new candidate disease gene on ch.17q11 in MS, and suggest that variation within NOS2A or a nearby locus contributes to disease susceptibility, and may also interact with HLA-DR.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Association of the homeodomain transcription factor *ENGRAILED 2* with autism spectrum disorder. *N. Gharani*[1], *R. Benayed*[2], *L.M. Brzustowicz*[1,4], *J.H. Millonig*[1,2,3]. 1) Dept Genetics, Rutgers Univ, Piscataway, NJ; 2) Center for Advanced Biotechnology and Medicine; 3) Dept Neuroscience and Cell Biology; 4) Dept Psychiatry, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.

Autism is a complex behavioral disorder that has a genetic and neurodevelopmental basis. Imaging and autopsy studies have demonstrated that the cerebellum is often maldeveloped in autistic individuals. The *Engrailed2-/- (En2)* mouse mutant displays similar cerebellar phenotypes. Human *EN2* maps to chromosome 7q36.3, a region that has demonstrated linkage to autism spectrum disorder in two studies. To examine whether *EN2* acts as a susceptibility locus, transmission/disequilibrium tests (TDT) were performed for two intronic SNPs (*rs1861972* and *rs1861973*), using the AGRE families. This dataset consists of small nuclear families with at least two members diagnosed with autism, Aspergers syndrome or Pervasive Development Disorder (PDD). For the purpose of our analysis a narrow diagnosis was defined as autism alone and a broad diagnosis included autism, Aspergers syndrome and PDD. Initially, TDT analysis was carried out on 137 unrelated trios of autistic individuals and their parents using the TRANSMIT program. Significant overtransmission of the A allele of *rs1861972* and the C allele of *rs1861973* was observed (*rs1861972* P=0.0009; *rs1861973* P=0.0006). Haplotype analysis revealed that the A,C haplotype is specifically overtransmitted in autistic individuals (P=0.000062). The analysis was then extended to include other affected and unaffected siblings as well as 29 additional families. The inheritance of each SNP was then assessed in these extended pedigrees using the Pedigree Disequilibrium Test (PDT). Significant association was once again observed for both SNPs under both the broad and narrow diagnostic schemes (*rs1861972*: narrow P=0.0080, broad P=0.0078; *rs1861973*: narrow P=0.0026, broad P=0.0090). Haplotype analysis on this extended sample confirms the overtransmission of the A,C haplotype (narrow P=0.0002, broad P=0.0004). In summary, these data identify *EN2* as a possible susceptibility locus for autism and related autism spectrum disorders.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 108 from 2003 ASHG Annual Meeting

Linkage to the AUTS1 locus and analysis of candidate genes for autism on chromosome 7q. *E. Bonora[1], J.A. Lamb[1], A. Abbott[1], N. Redhead[1], I. Ragoussis[1], A.J. Bailey[2], A.P. Monaco[1], International Molecular Genetic Study of Autism Consortium (IMGSAC)[3].* 1) The Wellcome Trust Centre for Human Genetics, University of Oxford, UK; 2) Department of Psychiatry, Warneford Hospital, University of Oxford, UK; 3) http://www.well.ox.ac.uk/~maestrin/iat.html.

Autism is a neurodevelopmental disorder of unknown etiology. Twin and family studies suggest a complex genetic predisposition to autism. Linkage and cytogenetic studies point to the presence of a susceptibility locus on chromosome 7q. Analysis of the expanded IMGSAC collection (219 ASP) reinforces support for linkage at this locus generating a multipoint MLS of 2.44 between D7S530 and D7S640. This linkage is driven entirely by the 144 male ASP (MLS of 2.83 between D7S480 and D7S530, compared to MLS of 0.12 in the 75 non-male ASP). Analysis of the parental contribution results in a paternal MLS of 2.20 between D7S477 and D7S2453, and a maternal MLS of 2.00 between D7S480 and D7S530. These data improve localisation of the linkage signal and support the presence of two discrete loci underlying the linkage to chromosome 7, with possible parent-of-origin effects.A subset of 48 autistic individuals was selected based on parental IBD sharing in the region and targeted screening of positional candidate genes was performed. Six genes, CUTL1, SRPK2, SYPL, NrCAM, LAMB1 and PTPRZ1, with a role in brain development and function, were analysed.43 changes in translated regions and 63 changes in promoter or intronic regions were detected, the most interesting being an aminoacid deletion in the homeobox domain of CUTL1 and 3 missense changes in LAMB1. The deletion in CUTL1 was not found in 192 controls and could not be identified in other autistic individuals. Two variants in LAMB1 were present at similar frequency in controls, whereas the exon 30 variant was more frequent in the autistic group (10.4% vs 3.7%). The missense changes, together with the most informative SNPs identified through our screen are currently being tested for association in the total IMGSAC collection, to investigate the possibility that the remaining DNA changes could play a role in autism susceptibility by subtly affecting gene expression or regulation.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 109 from 2003 ASHG Annual Meeting

Linkage disequilibrium mapping of a schizophrenia susceptibility locus on 1q22. *L.M. Brzustowicz*[1,2], *J. Simone*[1], *P. Mohseni*[1], *J.E. Hayter*[1], *E.W.C. Chow*[3], *A.S. Bassett*[3]. 1) Dept of Genetics, Rutgers University, Piscataway, NJ; 2) Dept of Psychiatry, UMDNJ, Robert Wood Johnson Medical School, Piscataway, NJ; 3) Dept of Psychiatry, University of Toronto, and Schizophrenia Research Program, Queen Street Division, Centre for Addiction and Mental Health, Toronto, Ontario.

We have previously reported linkage of schizophrenia to chromosome 1q21-22 with a maximum heterogeneity lod (HLOD) score of 6.50 in a group of 22 medium-sized multiplex Canadian families. Fine linkage mapping in the same sample has defined a Zmax-1 support interval of ~1Mb. We have now further investigated this region for evidence of linkage disequilibrium (LD). Evidence for LD was assessed using the program PSEUDOMARKER which, in contrast to popular TDT-based programs, explicitly separates the evidence for linkage and LD in families with multiple affecteds. Analyses were conducted using a recessive pseudomarker model, as there is significantly stronger evidence for a recessive mode of action for this locus in this sample, and using a narrow definition of affection (schizophrenia or schizoaffective disorder). Analysis of 10 microsatellite markers from a 1.6 Mb region produced significant evidence for LD given linkage at D1S2675 ($p=0.014$) and a CA-repeat ~40 kb proximal to D1S2675 ($p=0.023$). Analysis of 13 SNP markers located within 300 kb surrounding these 2 microsatellites identified 5 markers with significant evidence ($p<0.05$) of LD. Two adjacent SNPs, located 52 kb apart and flanking the significant microsatellite markers, were significant at $p<0.005$. All 7 markers in significant LD lie within the large second intron of the gene for CAPON, a brain-specific protein involved in signal-transduction of the NMDA receptor. Extensive screening of the coding sequence of CAPON has not identified any schizophrenia-associated variants, although the large genomic extent of this gene (>300 kb) presents a challenge to the exhaustive analysis of potential regulatory variants. These results suggest that a yet to be identified variant in CAPON, or another transcribed sequence within a large intron of this gene, appears likely to be implicated in susceptibility to familial schizophrenia linked to 1q22.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 110 from 2003 ASHG Annual Meeting

A combined population and family-based study of eight European populations demonstrates association between BDNF and eating disorders. *M. Ribases[1], M. Gratacos[1], F. Fernandez-Aranda[2], E. Cellini[3], B. Nacmias[3], S. Sorbi[3], D. Di Bella[4], L. Bellodi[4], P. Gorwood[5], A. Kipman[5], M.C. Mouren-Simeoni[5], J. Hebebrand[6], A. Hinney[6], H. Remschmidt[6], A. Karwautz[7], M. Brecejl[8], M. Gabrovsek[8], D. Collier[9], J. Treasure[9], X. Estivill[1], EC Framework V "Factors in Healthy Eating" consortium.* 1) Genomics Regulation Center, Barcelona, Spain; 2) Princes of Spain Hospital, Barcelona, Spain; 3) University of Florence, Florence, Italy; 4) Fondazione Centro S Raffaele, Milan, Italy; 5) Assistance-Publique des Hopitaux de Paris, France; 6) Philipps University Marburg, Germany; 7) University Clinic of Neuropsychiatry of Childhood and Adolescence, Vienna, Austria; 8) Department for Mental Health, Ljubljana, Slovenia; 9) Institute of Psychiatry, London, UK.

Animal models and genetic association studies in humans suggest that BDNF is a strong candidate for eating disorders (ED). We analyzed the 270C/T and the Val66Met SNPs within BDNF by a combined population and family-based study of independent recruited samples from eight European centers. We also analyzed the effect of these two SNPs in ED-related phenotypes such as minimum body mass index (minBMI), maximum body mass index (maxBMI), and age at onset of weight loss (AO). We performed a casecontrol study in 1142 patients with ANR, binge-eating/purging anorexia nervosa (ANBP) or bulimia nervosa (BN) and 510 sex-matched unrelated controls. We detected heterogeneity among populations and clinical subtypes. Once populations were analyzed together, we found that Met66 was strong associated to all ED subtypes [AN (P=0.002), ANR (P=0.009), ANBP (P=0.005) and BN (P=0.001)]. The multi-allelic version of the TDT approach also revealed an excess of transmission of the 270C/Met66 haplotype to the affected offspring in the AN (P=0.009) and ANR (P=0.007) groups. We also detected an effect of the 270C allele in old AO when BN patients were considered (P=0.001). These results support our initial hypothesis of a BDNF participation as a susceptibility factor to ED. To our knowledge, this is the first study that identifies a possible relevant gene involved in ED in different populations.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Positional cloning of candidate genes for developmental dyslexia=. *J. Kere[1,2], M. Taipale[2], N. Kaminen[2], J. Nopola-Hemmi[2,3], H. Anthoni[1], T. Haltia[4], B. Myllyluoma[2], H. Lyytinen[5], K. Muller[5], M. Kaaranen[5], P.J. Lindsberg[6], M. Peyrard-Janvid[1], K. Hannula[2].* 1) Department of Biosciences at Novum and Clinical Research Centre, Karolinska Institutet, Sweden; 2) Department of Medical Genetics, University of Helsinki, Finland; 3) Department of Pediatric Neurology, University of Helsinki, Finland; 4) Institute of Biomedicine/Biochemistry, University of Helsinki, Finland,; 5) Department of Psychology and Child Research Centre, University of Jyvaskla, Finland; 6) Department of Neurology, Helsinki University Central Hospital, Helsinki, Finland.

Approximately 3-10% of people have specific difficulties in reading, despite adequate intelligence, education and opportunity. We have positionally cloned and characterized a novel gene, DYX1C1 near the DYX1 locus in chromosome 15q21, that is disrupted by a translocation t(2;15)(q11;q21) segregating coincidentally with dyslexia (Taipale et al., PNAS in press). Two sequence changes in DYX1C1 associate alone and in combination with dyslexia. One involves the translation initiation sequence and an Elk-1 transcription factor binding site and the other introduces a premature stop codon, truncating the predicted protein by 4 amino acids. DYX1C1 encodes a 420-amino acid protein with three tetratricopeptide repeat domains. Comparison of human and nonhuman primates revealed relatively rapid recent evolutionary change for DYX1C1. DYX1C1 protein resides in the nucleus. In human brain, DYX1C1 protein localizes to a fraction of cortical neurons and white matter glial cells. In another branch of the project, we obtained evidence for haplotype association to dyslexia near the DYX3 locus on chromosome 2p in Finnish subjects (Anthoni et al., this meeting). Positional cloning with increasingly dense sets of SNP markers is underway. The identification of dyslexia genes may open a path to understanding a complex process of development and maturation of the human brain.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 112 from 2003 ASHG Annual Meeting

Evidence of interaction between susceptibility loci on chromosomes 1, 10 and 12 in late-onset Alzheimers disease (LOAD). *P. Holmans*[1,3], *A. Myers*[4], *J. Hardy*[4], *A. Goate*[2], *J. Williams*[3], *M. Owen*[3]. 1) MRC Biostatistics Unit, Inst Public Health, Cambridge, UK; 2) Washington University School of Medicine, St Louis, USA; 3) University of Wales College of Medicine, Cardiff, UK; 4) Laboratory of Neurogenetics, NIA,Bethesda, USA.

Myers et al. (2002) performed a genome scan for linkage on a sample of Caucasian LOAD families (450 sib pairs), finding a genome-wide significant peak on chr 10 (lod=3.9) and suggestive peaks (lods 1.0-1.9) on chr 1,5,6,9,12 and 21. Since it is likely that several loci are involved in the aetiology of LOAD, and that interactions between these loci may be important, the peaks on chr 1,5,6,9,12 and 21 were re-analysed allowing for potential interactions with a susceptibility locus on chr 10. This was done by modeling the ibd probability of each affected sib pair as a logistic regression on the proportion of alleles shared by that pair on chr 10. APOE genotype was included in the regression, as were APOExChr10 interaction terms (thereby allowing the interaction between the test locus and chr 10 to vary with APOE). Point-wise significant effects of the terms involving chr 10 were observed on chr 1,5,6,9 and 12. The APOE subgroups in which the chr 10 interaction was greatest were analysed separately, with a region-wide p-value for the interaction obtained by randomly permuting the chr 10 ibd scores among the sib pairs.

A significant effect was found on chr 12p, close to the A2M gene, in the 77 pairs where neither member had an APOE 4 allele, a lod of 1.35 being increased to 4.51 (region-wide p=0.004). The correlation in ibd with chr10 was negative, consistent with heterogeneity. Significant effects were also found on chr 1q, near marker D1S2345. In the 275 pairs where both members had an 4 allele, the lod increased from 1.03 to 3.17 (region-wide p=0.045), again with negative ibd correlation.In the remaining 165 pairs, the lod increased from 0.005 to 2.23 (region-wide p=0.05), with a positive ibd correlation (consistent with epistasis). These results suggest susceptibility genes for LOAD on chr 1, 10 and 12, interacting with each other and APOE.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 113 from 2003 ASHG Annual Meeting

Triplication of the normal alpha synuclein gene is a cause of hereditary Parkinson's disease. *A. Singleton*[1], *M. Farrer*[2], *J. Johnson*[1], *A. Singleton*[3], *S. Hague*[1], *J. Kachergus*[2], *M. Hulihan*[2], *T. Peuralinna*[1], *A. Dutra*[4], *S. Lincoln*[2], *A. Crawley*[3], *M. Hanson*[5], *M. Cookson*[6], *M. Muenter*[7], *M. Baptista*[6], *D. Miller*[6], *J. Blancato*[8], *J. Hardy*[5], *K. Gwinn-Hardy*[3]. 1) Molecular Genetics Section, Laboratory of Neurogenetics, National Institute on Aging, Bethesda, MD; 2) Genetics of Neurodegeneration Laboratory, Department of Neuroscience, Mayo Clinic, Jacksonville, FL; 3) Neurogenetics Branch, National Institute of Neurological Disorders and Stroke, Bethesda, MD; 4) Cytogenetic and Confocal Microscopy Core, National Human Genome Research Institute, Bethesda MD; 5) Laboratory of Neurogenetics, National Institute on Aging, Bethesda, MD; 6) Cell Biology and Gene Expression Section, Laboratory of Neurogenetics, National Institute on Aging, Bethesda, MD; 7) Mayo Clinic Scottsdale, Scottsdale AZ; 8) Department of Oncology, Georgetown University Medical Center, Washington, DC.

Mutations in the -synuclein gene are known to be a rare cause of early-onset Parkinsons disease (PD). We have been following a large family, the Iowan kindred with autosomal dominant disease. A genome wide screen was undertaken, including markers at the -synuclein locus that revealed a haplotype co-segregating with disease. Sequencing of -synuclein failed to reveal a pathogenic mutation and the identification of heterozygous single nucleotide polymorphisms ruled out a deletion as the causal event. Analysis of mRNA revealed one transcript of normal length and sequence. Examination of intragenic markers at the -synuclein locus was indicative of multiple alleles suggesting non-mendelian inheritance or a genetic multiplication event. This was confirmed by real time PCR amplification of each of the -synuclein exons in affected members of the kindred. This identified a whole gene triplication event; an observation that was confirmed using fluorescent *in situ* hybridization of chromosomes from EBV, immortalized lymphocytes. We have demonstrated the mutation results in an approximate doubling of the -synuclein protein in blood of an affected individual. This is the first described biomarker for PD and the first mutation that provides a direct mechanistic insight into the pathogenesis of PD.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 114 from 2003 ASHG Annual Meeting

Microsatellite polymorphism of Apolipoprotein C-II gene modifies age-at-onset of sporadic late-onset Alzheimer's disease independently of Apolipoprotein E-4 allele. *K. Kamino[1], T. Kida[1], T. Tanaka[1], T. Kudo[1], M. Yamamoto[1], H. Yamagata[2], T. Miki[2], M. Takeda[1]*. 1) Div of Psychiatry and Behavioral Proteomics, Dept of Post-Genomics and Diseases, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; 2) Dept of Geriatric Medicine, Ehime University School of Medicine, Onsen-gun, Ehime, Japan.

The genome-wide scanning of late-onset Alzheimer's disease (LOAD) has been noted that a region (19q13.2) close to apolipoprotein E (APOE) gene is a strong locus susceptible for and modifying age-at-onset of LOAD. Although the risk effect of the APOE-4 allele on the occurrence of LOAD has been well demonstrated, the effect of age-at-onset of the APOE-4 allele was not reproducible among patients with LOAD. In 190 Japanese patients with LOAD, we found that the allelic total of the microsatellite (GT)m(GA)n-repeat number encoded in intron 1 of apolipoprotein C-II (APOC2) gene is significantly correlated with age-at-onset of LOAD (p=0.0001). Patients with lower doses of the GT-repeat showed an earlier age-at-onset of LOAD compared to those with higher doses, and this effect was prominent in LOAD carrying APOE-4 allele. This microsatellite is not in linkage disequilibrium with the APOE genotype, and multiple regressions estimated that the (GT)(GA)-repeat dose of the APOC2 gene explains 27.7 percent of the variance of the age-at-onset, while the dose of APOE-4 does 6.8 percent. Thus, the APOC2 as well as the APOE gene also participates in the development of LOAD. It was noted that the first introns within most genes play important roles to control their transcription. Therefore, this microsatellite likely modifies the expressional level of apolipoprotein C-II. Since the APOC2 gene encodes a cofactor to activate lipoprotein lipase, relating to clearance of plasma chylomicrons that deliver fatty acid, our finding indicates that fatty acid metabolism etiologically influences the development of LOAD.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Human chromosome 1; finished sequence and transcript analysis. *S.G. Gregory.* Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, UK.

The production of high quality annotated sequence of chromosome 1, by the Wellcome Trust Sanger Institute, will provide a tremendous resource for the elucidation of disease-causing genes. The sequence will also form the basis for identifying genes linked with human cancer arising from alterations of chromosome 1, amongst the most common of genomic rearrangements associated with neoplasia. Here we present the generation and sequence analysis of chromosome 1 which constitutes an estimated 9% of the 2,800Mb human genome and includes 222Mb that contains transcriptionally active sequences (euchromatin). Chromosome 1 is the largest human chromosome (236Mb), is submetacentric and contains a large block of highly repetitive heterochromatin adjacent to the centromere on the q-arm. At the time of writing, the sequence ready map of chromosome 1 is contained within 20 bacterial clone contigs from which 2149 minimum tile path clones, representing 97% of the euchromatic region of the chromosome, have been sequenced to the finished standard. Gaps between contigs are estimated to be 6Mb in size based on fibre FISH or comparative sequence alignment. Where possible, gaps will be closed by screening genomic libraries containing 70 genome equivalents. Assessment of the integrity of the map and sequence is based upon; the identification of all known genes and markers contained within chromosome 1, correlation between in silico restriction fingerprints from finished sequence and the mapped clone, and determination of levels of sequence variation between clone overlaps. Genomic sequence analysis incorporates both in silico gene prediction and experimental homology screening. Chromosome 1 contains a total of 1985 known genes (full length protein sequences available in public sequence databases) and 464 novel genes (predicted on the basis of similarity to protein or cDNA sequences &/or ESTs, but which could not be mapped with confidence to existing entries). Of the 157 OMIM diseases currently known to localize to chromosome 1, 82% are found as full-length genes within the finished sequence. The remaining 18% are, at the time of writing, either partially present within finished sequence (15%) or currently missing from the finished assembly (3%).

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 116 from 2003 ASHG Annual Meeting

Development of a comparative functional approach to identifying mutations in non-coding sequences. *E. Grice, A. Chakravarti, N. Marsh-Armstrong, A.S. McCallion.* McKusick - Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

Mutations in non-coding, putatively regulatory sequences are often proposed as an explanation for the failure to identify coding sequence mutations in known disease genes. The identity and nature of such disease-causing mutations in non-coding sequence is unknown but comparative genomic analysis can be used to uncover the functional significance of conserved non-coding sequences. We have focused on Hirschsprung disease (HSCR) as an example of a multigenic disorder in which non-coding mutations likely exist. Mutations in the *RET* gene have been implicated in as many as 90% of familial HSCR, yet one half of the HSCR families demonstrating linkage to *RET* lack an identified coding sequence mutation. To facilitate simultaneous functional interrogation of the many conserved non-coding sequence elements at *RET*, we have utilized transgenesis in *Xenopus*. Using homologous recombination in bacteria, we introduced a GFP reporter in frame with the *RET* coding sequence contained in a 200 kb BAC. When placed in *Xenopus*, this BAC faithfully recapitulates the neural and renal expression of the human gene during development. As human genetic analyses have implicated sequence within the first intron of RET in HSCR susceptibility (See McCallion et al), we have established a panel of recombinasemediated mutations, deleting conserved elements within intron 1 and flanking the *RET* gene in BACs. Expression of RET:GFP in transgenic *Xenopus* harboring the panel of deletion constructs and in transgenic mice harboring the first of these RET:GFP BACs are currently being analyzed. These and subsequent analyses will identify the regulatory/functional non-coding elements predicted to harbor disease causing variants, and in turn add significant biological value to the expanding genome sequence data sets.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 117 from 2003 ASHG Annual Meeting

Comparative analysis of chimpanzee chromosome 22 and human chromosome 21: towards understanding human-specific characteristics. *T.D. Taylor, The Chimpanzee Chromosome 22 Sequencing Consortium.* RIKEN GSC, Yokohama, Japan.

To identify biologically and evolutionally important information acquired in the human lineage during the last 5-6 million years since the divergence from our closest ancestor the chimpanzee, we formed an international consortium to sequence chimp chromosome 22, the equivalent of human chromosome 21. We chose a clone-based sequencing strategy versus a whole-genome shotgun approach, since we could quickly establish a minimal tiling path of clones spanning most of chimp chromosome 22q using previously generated BAC-end sequence data. With some additional screening and walking we were able to cover the entire long arm except for four small gaps: two internal gaps which correspond to gaps in human chr21, and the regions near the centromere and telomere. In total, we determined 32.7 Mb of high-quality finished data. In order to avoid false-positive differences and to identify even the most subtle changes that may be significant between the chimp and human sequences, we felt that it was necessary to use such high-quality data for our analysis. How one defines similarity between species is a subjective matter. From our analysis, if we look at the base substitution rate between human and chimp, then we are 98.3% similar. If we take into account larger insertions and deletions, then the similarity drops to 94-95%. No large chromosomal rearrangements between the two sequences were observed. At the gene level, human and chimp are equivalent, except for one pseudogene missing in chimp. Several species-specific differences that may have an influence on expression or function were found within a subset of genes. We also identified several differences that may have an affect on DNA binding or regulation in the upstream promoter regions. Preliminary gene-profiling experiments have found significantly different levels of expression of a few genes in liver and brain between chimp and human. While comparisons with more distant species such as mouse are important for identifying conserved functional elements between our genomes, only by looking at the most closely-related species can we identity human-specific elements, which are essential for understanding our genome.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 118 from 2003 ASHG Annual Meeting

Global Mapping of Functionally-Important and Regulatory Regions on Human Chromosomes 21 and 22 Reveal Novel Regulatory Networks in the Human Genome. *D. Kampa[1], P. Kapranov[1], S. Cawley[1], S. Bekiranov[1], H.H. Ng[2], E.A. Sekinger[2], A. Piccolboni[1], V. Sementchenko[1], J. Cheng[1], J. Drenkow[1], M. Yamanaka[1], S. Patel[1], S. Brubaker[1], H. Tammana[1], B. Narayanan[1], G. Helt[1], K. Struhl[2], T.R. Gingeras[1].* 1) Affymetrix Inc, 3380 Central Expressway, Santa Clara, CA 95051; 2) Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115.

We recently reported up to an order of magnitude more transcriptional activity in human chromosomes 21 and 22 than previously anticipated based on the best-characterized subset of human annotations (Kapranov et al. (2002) Science 296, 916-9). To further explore properties of the human transcriptome, transcription factor binding sites (TFBS) for two common (Sp1, cMyc) and one specialized (p53) transcription factors have been mapped along the non-repetitive sequences of chromosomes 21 and 22 using a combination of chromatin immunoprecipitation and high-density oligonucleotide arrays. Such a comprehensive mapping approach helps both to provide anchor points to the start of individual transcripts and to assist in sorting out complex patterns of overlapping transcripts. Overall, an unexpectedly high count of TFBSs was found. The majority of TFBSs were found in non-canonical locations, such as internal, 3 or outside of the annotated genes. This data is consistent with the previously observed large-scale transcriptional activity in the human genome and points to possible function of novel transcripts. The combination of unbiased global mapping of transcribed and regulatory regions points to the hidden complexities of the regulatory landscape of the human genome.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 119 from 2003 ASHG Annual Meeting

Non-coding sequences that are conserved between humans and a limited number of mammals frequently behave as regulatory elements. *H. Tao[1], M.F. Doherty[1], X. Chen[1], K. Osoegawa[2], P.J. de Jong[2], D.R. Cox[1], K.A. Frazer[1]*. 1) Perlegen Sciences, Mt. View, CA; 2) Children's Hospital and Research Center, Oakland, CA.

Comparing human DNA sequences with those of different species is a fundamental method for identifying elements with biological importance due to the fact that functional sequences are conserved through evolution whereas non-functional sequences drift. A recent genome-wide comparison of human and mouse DNA discovered greater than 200,000 conserved non-coding sequences with unknown function. Multi-species DNA comparisons have been proposed as a method to prioritize this large number of conserved non-coding sequences for functional analysis based on the hypothesis that elements present in many species are the most likely to be functional. Here we perform a comparative analysis of the single-minded 2 (*SIM2*) gene interval on human chromosome 21 with horse, cow, pig, dog, cat, and mouse DNA. We classify conserved sequences based on the number of mammals in which they are present and test sequences in each class for function using two different experimental methods (transient transfection analyses and gel retardation assays). We demonstrate that the number of mammals a non-coding elements is present within is poorly correlated with its functionality (elements conserved in a limited number of mammals are just as likely to have function as elements conserved in many mammals) based on these two independent in vitro assays. Our data suggest that at least some of the non-coding elements that are conserved in only a limited number of mammals may play a role in gene expression differences between species. Furthermore, these data indicate that the expression of *SIM2* in humans may be controlled by the cumulative effects of numerous conserved non-coding elements.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 120 from 2003 ASHG Annual Meeting

Performance of DNA pooling across twelve large-scale association studies. *M.R. Nelson, S. Kammerer, M. Langdown, S. Mah, G. Marnellos, R. Roth, C.R. Hoyal, A. Braun.* Sequenom, Inc., San Diego, CA.

The growing catalog of known single nucleotide polymorphisms (SNPs) and the technological advances for measuring them on a large scale are improving the prospects for conducting genome-wide association studies. However, with the costs of individual genotyping still relatively high, we have implemented a strategy of association that utilizes DNA pools to estimate allele frequencies for cases and controls to identify genes associated with disease. The strategy consists of three steps: 1) testing all SNPs in pools with minimal replication; 2) re-testing SNPs that show evidence of association with additional replication at key steps in the measurement process; and 3) the most strongly associated SNPs are genotyped on every individual included in the DNA pools to confirm the association. This strategy requires approximately 100 times fewer measurements than individually genotyping all SNPs. To date we have conducted 12 large-scale association studies using this strategy. The pooled allele frequencies were estimated with chip-based MALDI-TOF mass spectrometry for 28,000 to 85,000 SNPs located in gene regions throughout the genome. Incorporating the estimates of technology variation into the analysis of pooled data yields the expected fraction of tests with p-values less than 0.05 and 0.01, but a larger than expected fraction of tests with smaller p-values. Over 1,000 SNPs were selected from the pooled comparisons for these studies that were subsequently genotyped for each individual. Significance tests from analyses of pooled data agree with those from individual genotypes for 55% to 80% of genotyped SNPs across studies. The bias of pooled allele frequency estimates due to preferential PCR amplification, primer extension competition, and differential analyte sensitivity in mass spectrometry measurement had a minor impact on study conclusions. While the variability introduced by DNA pooling decreases statistical power, our experience supports its use as a cost-effective alternative for conducting genome-wide association studies to identify genetic variations that influence common disease susceptibility.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 121 from 2003 ASHG Annual Meeting

Pool-based association scan for type 2 diabetes using 49,419 SNPs in a 92.2 Mb region on chromosome 6. *L.J. Scott*[1], *A.B. Sparks*[2], *K.L. Mohlke*[3], *D.A. Hinds*[2], *M. Li*[1], *D.G. Ballinger*[2], *P.S. Chines*[3], *J. Tuomilehto*[4], *R.N. Bergman*[5], *K.A. Frazer*[2], *R.M. Watanabe*[5], *F.S. Collins*[3], *D.R. Cox*[2], *M. Boehnke*[1]. 1) U. Michigan, Ann Arbor, MI; 2) Perlegen Sciences, Mountain View, CA; 3) NHGRI, Bethesda, MD; 4) Nat'l Public Health Inst., Helsinki, Finland; 5) U. Southern California, Los Angeles, CA.

The aim of the Finland-United States Investigation of NIDDM Genetics (FUSION) Study is to identify genetic variants that contribute to development of type 2 diabetes (T2D). Based on 735 FUSION families, we identified overlapping linkage signals in a 90 cM (92.2 Mb) region of 6q. These included signals for T2D (LOD=2.66 at 95.5 cM), for the subset of T2D families with highest mean HDL/total cholesterol ratio (LOD=7.93 at 78.0 cM), and for fasting insulin QTL (LOD=2.65 at 139.0 cM). To scan for diabetes-associated variants, we assayed 49,419 SNPs in the 92.2 Mb interval (1.8 kb average density) using Perlegen's high-density oligonucleotide array genotyping platform. Eight replicate measurements were collected for each SNP from each of 3 pools: 464 T2D cases, 152 HDL ratio cases, and 205 elderly non-diabetic controls. Indirect estimates of case-control allele frequency differences were calculated from the array data for each of the 47,269 SNPs (96% of the total) that passed QC criteria (low replicate variability, n>5). Perlegen's reference haplotype map was used to identify SNPs that occur in the same haplotype block, and are therefore expected to provide concordant information. We selected 624 SNPs for individual genotyping by defining thresholds for estimated allele frequency differences, with lower thresholds for SNPs closer to a linkage peak, within relevant portions of genes, or with concordant haplotype block information. For redundant SNPs in the same haplotype, only the SNP with the largest estimated allele frequency difference was selected. Genotyping of the individuals in the pools is underway to evaluate the selection criteria and to identify SNPs with significant allele frequency differences for follow-up genotyping in this and other populations.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 122 from 2003 ASHG Annual Meeting

Whole genome scan in a complex disease using 11,245 SNPs. *G. Kennedy[1], N. Shephard[2], M. Cao[1], J. Chen[1], N. Vasavda[3], G. Liu[1], T. Mills[3], N. Gibson[3], K. Jones[1], J. Worthington[2], S. John[2].* 1) Dept Genotyping Research, Affymetrix, Santa Clara, CA; 2) University of Manchester, Manchester, England; 3) Astrazeneca, Alderley Park, England.

Genome scans comprising ~400 microsatellite markers have been applied successfully to linkage studies; however genotyping microsatellites is resource intensive. It has been proposed that linkage analysis with a set of high density SNPs could achieve equivalent information content to microsatellites. We used a recently described array-based genotyping technology called whole genome sampling analysis (WGSA), which uses one generic primer to amplify >10,000 SNPs in a single reaction and makes automated genotype calls at >99% accuracy. Using this technology, we undertook a paradigm study to determine whether high density SNP markers could be used successfully in a genome scan for a complex disease. We chose rheumatoid arthritis (RA), a chronic inflammatory disease in which 4 microsatellite whole genome scans have been published. We analyzed 655 individuals, the UK cohort of 252 affected sibling pairs(MacKay 2002), for 11,245 genome-wide SNPs and obtained >6.6 million genotype calls. The median spacing of the SNPs is 115 kb and the median heterozygosity is 0.40. Non parametric multi-point linkage analysis was performed using Merlin (Abecasis 2001). In addition to confirming linkage to the HLA, we obtained evidence for linkage to six other regions, two were detected in the microsatellite whole genome scans and four were not. Three loci detected in the microsatellite genome scans were not detected in the current SNP study. Several factors could account for these results; differences in information content and marker density between microsatellites and SNPs, and possible linkage disequilibrium between SNPs. We calculated multipoint polymorphic information content and found it to be uniformly high (85-97%) across the genome. Due to the higher information content of the SNP based genome scan, peaks were generally better defined with a narrower 1 LOD interval. As WGSA technology is scalable to >100,000 SNPs, this approach has great potential utility in elucidating the genetic basis of complex human disease.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

A Survey of the Genomic Background of Low Frequency SNPs. *S.B. Gabriel[1], N. Patterson[1], D.E. Reich[1], A. Lochner[1], G. McDonald[1], J.M. Moore[1], J. Roy[1], S.F. Shaffner[1], M.J. Daly[1], D. Altshuler[1,2].* 1) Ctr Genome Research, Whitehead Inst, Cambridge, MA; 2) Departments of Genetics and Medicine, Harvard Medical School; Department of Molecular Biology , Mass General Hospital, Boston, MA.

Genetic variants that contribute significantly to human disease show extensive variation in allelic spectrum. Successful association studies will require a detailed understanding of the genetic structure underlying both rare and common alleles. Large-scale efforts are underway to characterize genetic variation across the genome through a map of common haplotypes. A critical question is whether LD based approaches can be practically applied to study alleles of low frequency and in particular those that act recessively. To address this question we surveyed several genomic regions deeply, in order to completely capture all variants, and broadly in order to have sufficient power to study the extent and diversity of haplotypes around low frequency SNPs. Twelve Mb regions were studied, each centered on a deeply resequenced core. Having resequencd the core, we ascertained all variant sites, including low frequency alleles(2-5%). To study LD around low frequency alleles, we genotyped large sample sets (400 chromosomes each of European and African samples) for SNPs in the core and common SNPs (1/25kb) across a Mb flanking the core. Initial analysis of haplotype diversity shows that low frequency alleles are mostly observed on a single ancestral haplotype, indicating that each is typically the result of a single mutational event in human history. We observe long-range haplotype homozygosity around these less frequent alleles, extending substantially further than around alleles >5%. We use this data to estimate power of association studies. Given modestly favorable models for genetic risk of disease, and particularly for recessive models, we find that reasonably sized case control studies using a framework of common SNPs would have substantial power to detect association to infrequent alleles. These data support that a map of common haplotypes will be useful for association mapping to variants of lower frequency and recessive inheritance, as well as common alleles as usually proposed.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 124 from 2003 ASHG Annual Meeting

Combinatorial mismatch scan for Successful Aging loci in the Amish. *W.K. Scott[1], P.C. Gaskell[1], C.E. Jackson[2], J.L. Haines[3], M.A. Pericak-Vance[1], E.R. Hauser[1].* 1) Duke University Medical Center, Durham, NC; 2) Scott & White, Temple, TX; 3) Vanderbilt University Medical Center, Nashville, TN.

Reports of significant heritability and linkage of extreme longevity to chromosome 4 have increased interest in finding genes positively influencing aging. Successful aging (SA) involves avoiding disease and disability, maintaining high cognitive and physical function, and being socially engaged throughout the lifespan. Combinatorial mismatch scanning (CMS; Heath et al. Hum Hered 2001) was proposed to detect IBD sharing in distantly related individuals from founder populations. The Amish communities of northeastern Indiana were founded in the 1850s by a small number of individuals and have remained isolated (coefficient of inbreeding=0.0195, parents between 1st and 2nd cousins). We enrolled 41 Amish over age 80 in a pilot study of SA. Data collected included family history, DNA, cognitive screening (MMSE), self-reported activities of daily living (ADL), and an objective test of physical function (balancing, walking, standing, foot taps, and writing). Nine subjects were successfully aged based on established cut-points for optimal functioning (MMSE>26, ADL=0, top 1/3 of sample on physical function tests). For CMS analysis, we chose 16 subjects with no common ancestors back to their grandparents and on whom genotypes at 380 autosomal microsatellite markers had been generated in a genomic screen for Alzheimer disease. To control for confounding by cognitive status, we chose subjects with MMSE>26. Mean age of the 8 SA 'cases' was 85.3 (6 women), and mean age of the 8 non-SA 'controls' was 88.5 (6 women). Case-control comparisons using Fisher's exact test identified significant ($p<0.05$) differences at 17 markers (4.5%). The most significant results were at D6S1007 ($p=0.0007$), D1S552 ($p=0.005$) and D8S272 ($p=0.007$). Three other regions (chromosomes 5, 6, and 14) produced significant results at two consecutive markers. These data suggest that one or more regions of the genome are associated with higher physical function in cognitively intact older Amish. Follow-up will include bootstrap analysis of allele-specific effects at these markers and additional genotyping in a larger sample.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Striking differentiation of sub-populations within a genetically homogeneous isolate (Ogliastra) in Sardinia as revealed by mtDNA analysis. *E. Petretto*[1,2], *C. Fraumene*[1,2], *A. Demontis*[3], *A. Angius*[1,2], *M. Pirastu*[1,2,3]. 1) Institute Population Genetics, CNR,SS,Italy; 2) SharDNA Life Science,CA,Italy; 3) Parco Genos,NU,Italy.

Sardinia is considered an isolate because of its strong genetic differentiation compared with European populations although a slight population structure is present within Sardinians. The Ogliastra population (~60.000 inhabitants), based in a remote Eastern Sardinia mountain area, is characterized by distinct genetic and linguistic features. We analyzed mtDNA haplogroups and HVS-I variation in 175 subjects from 20 out of the 23 Ogliastra villages. Ogliastra results as the most genetically homogenous (GDV=0.82) European population. HVS-I sequences are characterized by smaller than average number of polymorphic sites and a small number of distinct lineages (s=3.66,k=7.80,=2.38). The relative lack of variation in HVS-I is indicative of a major founder event, and for the H haplogroup we estimate that a demographic expansion occurred during the Neolithic (~7,700 YBP), as supported by archaeological studies. We reconstructed all the genealogies in 3 Ogliastra villages (Talana 1200, Perdasdefogu 2400, Urzulei 1400) for the last 400 years. For each of these, we chose 100 subjects that fully represented all maternal lines. In order to avoid unbalanced sampling, we selected subjects in proportion to the number of individuals belonging to each maternal line in present-day population. The analysis of mtDNA transmission in conjunction with maternal genealogy prove the high reliability of archival data. We observe a limited number of genetic lineages (from 15 to 22) present in the 3 villages. MtDNA differentiation in these villages versus Ogliastra as a whole, reveales a strong demarcation in their genetic pools due to distinctive founder effects. For instance in 2 villages, within the H haplogroup (~75%) we found distinctive expanded sub-haplogroups suggesting different ancestors contribution. Although Ogliastra can be considered a genetically homogeneous isolate, small villages divergent genetic histories underline the importance of a systematic analysis of DNA variation between and within populations.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Genetic variation in an isolated population, the Samoans of Polynesia: implications for mapping complex traits.

R. Deka[1], S.T. McGarvey[2], D.E. Weeks[3], S.R. Indugula[1], G. Zhang[1], H-J. Tsai[3], J. Akey[1], N. Wang[1], D. Smelser[1], P. Pal[1], R. Kaushal[1], G. Sun[1], S. Viali[4], J. Tufa[5], R. Chakraborty[1], L. Jin[1]. 1) Dept. Environmental Health, Univ. Cincinnati, OH; 2) International Health Institute, Brown University, Providence, RI; 3) Dept. Human Genetics, Univ. of Pittsburgh, PA; 4) Dept. Health, Apia, Samoa; 5) Dept. Health, Pago Pago, American Samoa.

The Samoan islands were settled by humans 3,000 years BP during the course of colonization of Polynesia. Archeological and ethnographic data suggest the pre-European contact population size at an estimated 100,000-300,000 individuals, which suffered a massive depopulation to 40,000 during the 19th century. This was followed by a population expansion during the past 100 years and the current Samoan population size is about 220,000. We present three sets of data on the extent of genetic variation in this population: (1) an analysis of 400 microsatellite markers distributed throughout the genome showing a significant reduction of genetic variation in this island population compared to continental populations; (2) a genome wide (381 autosomal + 18 X-chromosome markers) marker to marker linkage disequilibrium (LD) analysis, LD extending over large enough distances making it feasible for genome-wide association testing using microsatellite panel of markers; (3) an LD analysis using a set of high-resolution (2 kb) SNP markers on a 104 kb DNA fragment on chromosome 21 showing that the Samoans have significantly elevated levels of LD (minimum average D values 0.6) compared to four continental populations (Nigerian, German, Japanese and Chinese) throughout the 104 kb region without any sign of attenuation. The data further support the scenario of a recent population bottleneck followed by expansion, consistent with the known demographic history of the population. These results imply that a relatively large isolated population living in a homogeneous environment even with a relatively longer founding history (3,000 years) could be potentially useful in searching for genes associated with complex diseases. Supported by NIH grants AG09375, HL52611, DK55406, DK59642.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

MtDNA evidence for a genetic bottleneck in the early history of the Ashkenazi Jewish population. *M.F. Hammer[1], D. Garrigan[1], D.M. Behar[2], M.E. Kaplan[1], K. Skorecki[2].* 1) Division of Biotechnology, University of Arizona, Tucson, AZ; 2) Bruce Rappaport Faculty of Medicine and Research Institute, Technion and Rambam Medical Center, Haifa, Israel.

The evolutionary mechanisms responsible for increased frequencies of approximately 20 disease alleles in the Ashkenazi Jewish population remain controversial. Potential explanations include natural selection (i.e., heterozygote advantage) and accentuated genetic drift through recent and/or more ancient founder effects. To test for the effects of a bottleneck on the Ashkenazi Jewish population, we performed an extensive analysis of mtDNA HVS-1 sequence and restriction site polymorphisms in 565 Ashkenazi Jews from different parts of Europe. These patterns of variation were compared with those of 5 Near Eastern (n = 327) and 10 host European (n = 849) non-Jewish populations. Only four mtDNA haplogroups (defined on the basis of diagnostic coding region RFLPs and HVS-1 sequence variants) account for ~70% of Ashkenazi mtDNA variation. While several Ashkenazi Jewish mtDNA haplogroups appear to derive from the Near East, there is also evidence for a low level of introgression from host European non-Jewish populations. HVS-1 sequence analysis revealed increased frequencies of Ashkenazi Jewish haplotypes that are rare or absent in other populations, and a reduced number of singletons in the Ashkenazi Jewish sample. These diversity patterns provide evidence for a period of prolonged low effective size in the history of the Ashkenazi population. The data best fit a model of an early bottleneck (~100 generations ago), perhaps corresponding to initial migrations of ancestral Ashkenazim in the Near East or to Europe. A genetic bottleneck followed by the recent phenomenon of rapid population growth are likely to have produced the conditions that led to the high frequency of many genetic disease alleles in the Ashkenazi population.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Investigations of Male Founder Structure and the Y Chromosome in the Old Order Amish. *T.I. Pollin[1], R. Agarwala[2], A.A. Schäffer[2], A.R. Shuldiner[1], B.D. Mitchell[1], J.R. O'Connell[1].* 1) University of Maryland, Baltimore, MD; 2) National Center for Biotechnology Information, NIH, DHHS, Bethesda, MD.

Despite extensive genetic disease studies of the Old Order Amish of Lancaster County, PA, molecular confirmation of the founder size has yet to be reported. We used the Anabaptist Genealogical Database and the query software PedHunter to construct 28 all-male lineage pedigrees connecting the 739 males enrolled in our complex genetic trait studies and genotyped for up to 9 STR markers on the Y chromosome. Only 2 of the 28 lineages shared the same surname, and seven surnames accounted for 83% of all enrollees. We observed 22 distinct inferred founder haplotypes in the 23 families with all 9 markers typed; the 2 families with identical haplotypes had different surnames. We also studied deviations within each pedigree from the inferred founder haplotype to attempt to distinguish between genotyping error, pedigree/sample error and true mutations. After excluding 3 apparent pedigree/sample errors, 71(1.2%) of 5794 single-marker genotypes deviated from the apparent founder allele for a given lineage, with both gains and losses in repeat numbers observed. Fifty-four such cases clustering in sublineages (size 2 to 16 relatives) appeared to represent 12 unique historical mutation events. The remaining single-marker mutations appeared in 8 discordant father-son pairs and 9 sons with untyped fathers. Based on the discordant father-son pairs as a proportion of the total number of observed meioses per marker (n =119 to 283), the estimated marker-specific mutation rates ranged from 0 to 10.95×10^{-3} (average 3.99×10^{-3}) mutations/meiosis, similar to mutation rates reported by other investigators. These findings provide molecular confirmation of the male founder structure of our Amish study sample and insight into the mutation rates in Y-linked STR markers, which should enhance genetic disease research in this population. Future plans include confirming putative mutations, genotyping additional markers on the Y chromosome to further elucidate relationships between individuals/lineages and using mitochondrial markers to assess female founder structure.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Molecular genetics of two ancient Mediterranean populations. *J.R. Hughey*[1], *M. Michalodimitrakis*[2], *A. Vasilakis*[3], *E. Konsolaki*[4], *G. Stamatoyannopoulos*[1]. 1) University of Washington, Seattle, WA, USA; 2) University of Crete, Heraklion, Greece; 3) Heraklion Archeological Museum, Heraklion, Greece; 4) Piraeus Archeological Museum, Piraeus, Greece.

Of special interest to archeologists are populations involved in the establishment of ancient civilizations around the Mediterranean basin. One such population established the Minoan civilization on the island of Crete in the second and third millennia B.C. This civilization abruptly ended around 1450 B.C. after a cataclysmic volcanic eruption on Thera. The Mycenean civilization followed and had its own distinct cultural practices and writing. It is unknown whether the two civilizations are the product of different people or how these people are related to other mediterranean populations of the same period or the populations of classical Greece. To investigate these issues we have initiated genetic studies of these populations from various localities. So far we have collected material from two second millenium B.C. settlements in eastern Crete (belonging to 31 individuals), one settlement in central Crete (40 individuals), and one Mycenean settlement from the island of Salamis in Attica (30 individuals). The major problem in molecular archeological studies is contamination by exogenous DNA. In this study severe precautionary steps were taken to avoid contamination, including the use of teeth as the source of DNA. For each tooth, four PCR amplifications were performed. The targets were 100-150 bp overlapping amplicons that span the Hypervariable Sequence One (HVS 1) region of the Mitochondrial genome. Amplicons were cloned and eight colonies were sequenced. A consensus sequence for each tooth was determined from approximately 30 clones. So far we have analyzed the sequences from 27 Minoans from the two eastern Crete Burials and 12 Myceneans. Haplotype frequencies of the HVS 1 revealed considerable differences between the populations studied. Frequencies of the Minoans from central Crete and other Myceneans are currently being determined. Our results offer an illustration of how molecular techniques can provide insights into the genetics of human populations that lived four to five millenia ago.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 130 from 2003 ASHG Annual Meeting

Beyond summary statistics: human population genetic structure from the perspective of the individual. *J. Mountain.* Dept Anthropological Sci, Stanford Univ, Stanford, CA.

Descriptions of the genetic structure of our species typically rely on *a priori* assignment of individuals to populations. Population structure is then described in terms of within group variation (e.g. heterozygosity), between group variation (e.g., genetic distance), and other variance components (e.g. F_{ST}). Multilocus measures such as linkage disequilibrium provide an additional dimension, but again require *a priori* assignment of individuals to populations. Over the past decade geneticists have developed methods for summarizing human population genetic structure without such *a priori* assignment of individuals to groups. Using trees of individuals and cluster analyses derived from multilocus genotype data, researchers can now compare the inferred genetic structure with social or geographical groupings. Here I introduce a novel approach to the description of population genetic structure that focuses even more closely on the individual. I describe individual relatedness profiles derived from a set of 377 STR loci tested in over 1000 individuals of 52 human populations (CEPH diversity panel). These profiles vary in shape both within and across geographically and socially defined groups and reveal the inadequacy of the commonly used summary statistics. Relatedness profiles are informative both for the inference of human history and within genetic epidemiology, indicating, for instance, how well particular racial and ethnic labels serve as proxies for relatedness.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 131 from 2003 ASHG Annual Meeting

Out of Africa hypothesis supported by variation at CD4 and DM1. *N. Mukherjee, P. Paschou, M.M.C. DeMille, A.J. Pakstis, J.R. Kidd, K.K. Kidd.* Department of Genetics, Yale University School of Medicine, New Haven, CT 06520 USA.

The origin of modern humans (Homo sapiens sapiens) has long been an issue of controversy and debate. The Out-of-Africa (OOA) model has been supported by fossil records and genetic studies. CD4 and DM1 were among the first autosomal loci used to argue for the OOA model (Tishkoff et al., 1996; 1998) based on global patterns of haplotype frequencies and linkage disequilibrium (LD). We have now extended studies on these two loci compared to the earlier reports by adding 9 new population samples from Africa, East Asia, Europe, and North America and five new markers. At CD4 two new SNPs and an STRP extend coverage to a total of 38 kb while at DM1 two SNPs extend the region studied to 110 kb. Overall we studied 32 populations (1,484 individuals) and 11 polymorphic sites at CD4 and DM1. Analyses included estimation of multi-site haplotype frequencies plus pairwise as well as segmental and overall LD coefficients. African populations have greater diversity for both STRP alleles and haplotypes defined with SNPs. All the haplotypes and STRP alleles detected in the non-African populations are a subset of those found in African populations. A clinal gradient in haplotype frequency is seen from Africa to the Americas. At DM1 there is little or no LD present between the extreme markers (110kb) except in Native American populations, but for markers just a little closer at 108 kb apart, moderately strong LD is still detectable for some populations outside of Africa including some European population samples. For the original 30 kb region very strong LD exists everywhere. At CD4 the strong LD previously reported between the Alu deletion and pentanucleotide alleles (especially in non-African populations) extends to strong LD between the 3 SNPs and the two STRPs worldwide although the simple pairwise LD between the SNPs presents a more complex pattern. These results continue to support the OOA model. This additional information at these loci provides the potential for finer resolution of population relationships. (Supported in part by NIH GM57672 and NSF BCS96588.).

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 132 from 2003 ASHG Annual Meeting

Signatures of strong positive selection at the lactase gene. *T. Bersaglieri[1,2], J. Drake[1,2], T. Vanderploeg[1,2], P.C. Sabeti[3], D.E. Reich[3], J.N. Hirschhorn[1,2,3]*. 1) Genetics, Children's Hospital, Boston, MA; 2) Genetics, Harvard Med School, Boston, MA; 3) Center for Genome Research, Whitehead/MIT, Cambridge, MA.

Genes under positive selection provide insight into the recent evolutionary forces that have acted on human populations, and may also have medical implications (e.g. sickle cell disease). We looked for signatures of selection at the lactase gene (*LCT*), because persistence of lactase expression into adulthood is common in European-derived populations and rare elsewhere, perhaps due to selective advantage provided by the introduction of dairy farming in Europe. A variant 14 kb upstream of *LCT* has been strongly associated with lactase persistence (-13910C/T; Ennatah et al. 2002).

We genotyped 74 single nucleotide polymorphisms (SNPs) in 3 Mb around *LCT* in 60-96 chromosomes from 3 different populations: European-derived (CEPH), African-American, and East Asian (Chinese and Japanese). We looked for 3 signatures of selection: an excess of SNPs with high F_{ST}, a long haplotype present at high frequency (EHH; Sabeti et al. 2002), and a novel signature, long runs of SNPs with similar p_{excess} values.

75% of European, 14% of African-American, and none of the East Asian chromosomes carried the persistence allele (T) at 13910. Almost all of the chromosomes with this allele had an identical 42-marker 900 kb haplotype around *LCT*. Comparing this 900 kb region to the rest of the genome, there was a strongly significant excess of high F_{ST} values: 22 of 42 SNPs had F_{ST} values >0.2 (comparing US Caucasians with East Asians), and 3 SNPs, including 13910C/T, had values >0.5. The EHH test for this haplotype was strongly positive, as was the test for correlation in p_{excess}. Finally, using the markers flanking the 900 kb region, we estimate that this haplotype is only a few thousand years old.

Given the strength of the signatures of recent positive selection, and the very high frequency and young age of the haplotype in Europeans, our data suggest that *LCT* contains one of the strongest signatures of positive selection yet described in humans.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Analysis of DNA seqeunces and repeat lengths under unequal evolutionary rates. *J.C. Long, K. Hunley.* Dept Human Genetics, Univ Michigan Medical Sch, Ann Arbor, MI.

Many simple statistics for measuring genetic diversity, such as Fst, are of limited value because: (1) they are implicitly concerned with allelic identity by state and hence ignore the total number of nucleotide differences between sequences, and (2) they are biased by invalid assumptions, such as, all subpopulations have the same expected genetic diversity, and all subpopulations evolve independently. In order to relax these limitations, a linear statistical model and maximum likelihood estimation procedure is formulated that weights the probability that two copies of the locus will differ in state by the number of differences between them. The unique features of this model are that it allows each subpopulation to have its own level of genetic variation, and it allows a complex hierarchical structure for subpopulations. The model combines aspects of the statistical methods developed by Excoffier (Genetics, 131:479) and Urbanek (Mol Bio Evol, 13: 943).

The method is demonstrated here by performing an analysis to reveal the relative importance of population hierarchy, unequal diversity within subpopulations, and the number of mutational steps between alleles. Two samples of 16 populations with worldwide distribution were collected from web sources. The first consists of N=1006 mtDNA D-loop sequences and the second consists of N=378 individuals genotyped for 377 autosomal microsatellites. The first data set was analyzed according to the infinite sites mutation model while the second data set was analyzed according to a stepwise mutation model. This analysis reveals that assumptions made at one level of hierarchy (i.e., within subpopulations) can have large effects on estimates of parameters at other levels (i.e., among subpopulations). A surprising consequence of this is that Fst is always biased downwards. These findings suggest that the genetic diversity among human populations is greater, and more richly patterned, than heretofore appreciated.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 134 from 2003 ASHG Annual Meeting

Comparative linkage disequilibrium analysis of the -globin hotspot in primates. *J. Wall[1], L. Frisse[1], R. Hudson[2], A. Di Rienzo[1]*. 1) Human Genetics, University of Chicago, Chicago, IL; 2) Ecology and Evolution, University of Chicago, Chicago, IL.

Recombination rates vary both across the genome and between different species, but little information is available about the temporal and physical scales over which such rates change. In order to shed light onto these questions, we carried out a high resolution analysis of a genomic region within the -globin gene cluster known to experience elevated recombination rates in humans. To this purpose, we developed new linkage disequilibrium-based methods that thoroughly search for subsets of the data with unusually high (or low) estimated values of the population recombination parameter (4Nr, where N is the effective population size and r is the cross-over rate between adjacent base pairs). By re-sequencing a 15 kb segment in a human population sample, we were able to narrow down the recombinational hotspot to a segment less than 2 kb long which coincides with the -globin replication origin. In addition, we analyzed the orthologous region in samples of rhesus macaques and common chimpanzees. While the analysis of the chimpanzee data is complicated by the sample structure, the macaque data imply that this region may not be a hotspot in this species. These results suggest a time scale for the evolution of hotspots in primates. Furthermore, they allow us to propose diverged sequence elements that may contribute to the differences in the recombinational landscape in the two species.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Microarray-based identification of placenta-derived mRNA markers for detection in maternal plasma: towards non-invasive prediction of preeclampsia. *R.W.K. Chiu[1], N.B.Y. Tsui[1], S.S.C. Chim[1], E.K.O. Ng[1], Y.K. Tong[1], T.K. Lau[2], T.N. Leung[2], Y.M.D. Lo[1]*. 1) Departments of Chemical Pathology,and; 2) Obstetrics and Gynaecology, The Chinese University of Hong Kong, Hong Kong.

The presence of fetal nucleic acids in maternal plasma opened up the opportunity for non-invasive prenatal diagnosis. Aberrations in fetal DNA concentrations in maternal plasma have been reported for preeclampsia and fetal chromosomal aneuploidy. However, the analyses were based on the quantification of Y-chromosome-specific sequences, thus, limiting the applicability of such an approach to only 50% of all pregnancies. The recent demonstration of the presence of placenta-derived mRNA species in maternal plasma allows the development of gender-independent assays. In this study, we aimed to systematically identify a panel of placenta-specific mRNA species that could be robustly detected in maternal plasma. Gene expression profiles of placental tissues and corresponding maternal whole blood samples fromnormal pregnancies were assessed and compared by oligonucleotide microarray analysis (Affymetrix). Transcripts that were expressed in the placental tissue but not in maternal whole blood were ranked according to the expression levels. Real-time quantitative RT-PCR assays were designed for maternal plasma analysis of seven transcripts. Similar experiments were performed for preeclamptic pregnancies and controls. Panels of placenta-expressed mRNAs that were not expressed by the maternal hematological system were identified. The identity and ranking of the mRNA species within the panels differed between the first and third trimesters, and also differed between age-matched preeclamptic and normal pregnancies. The placental tissue expression levels of the seven transcripts, assessed by RT-PCR and microarray analyses, showed close correlation with one another. Remarkably, the expression patterns in maternal plasma determined by RT-PCR correlated closely with those of the placental tissues. In conclusion, this approach effectively identified a large panel of placenta-derived mRNA species that are detectable in maternal plasma, and thus paves the way for a generic approach of non-invasive prenatal diagnosis.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 136 from 2003 ASHG Annual Meeting

Non-invasive prenatal diagnosis of -thalassemia by mass spectrometric analysis of fetal DNA in maternal plasma.

C. Ding[1], R.W.K. Chiu[2], T.K. Lau[3], T.N. Leung[3], L.C. Chan[4], A.Y.Y. Chan[4], P. Charoenkwan[5], I.S.L. Ng[6], X.M. Xu[7], C. Wanapirak[5], T. Sanguansermsri[5], E.S. Ma[4], D.H.K. Chui[8], C.R. Cantor[1,9], Y.M.D. Lo[2]. 1) Bioinformatics Program and Center for Adv Biotech, Boston University, Boston, MA; 2) Departments of Chemical Pathology; 3) Obstetrics and Gynaecology, The Chinese University of Hong Kong, Hong Kong; 4) Division of Hematoloy, Department of Pathology, The University of Hong Kong, Hong Kong; 5) Faculty of Medicine, Chiang Mai University, Thailand; 6) Division of Genetics, Dept of Paediatrics, KK Women's and Children's Hospital, Singapore; 7) Department of Medical Genetics, First Military Medical University, Peoples Republic of China; 8) Boston University School of Medicine, Boston; 9) Sequenom, Inc., San Diego.

-thalassemia is one of the most common autosomal recessive diseases in the world. Non-invasive prenatal diagnosis based on the analysis of fetal DNA in maternal plasma can be performed safely using a single maternal blood sample. In this study, we evaluated the use of the MassARRAY system (Sequenom) for the robust detection of fetal DNA in maternal plasma for the prenatal diagnosis of -thalassemia. The MassARRAY system is designed for high throughput single nucleotide discrimination based on primer extension followed by MALDI-TOF mass spectrometry analysis. In the feasibility study, fetal gender was determined with maternal plasma DNA for 46 normal pregnancies. All of the 37 male and 9 female fetuses were correctly predicted. In addition, twenty-three couples undergoing prenatal diagnosis for -thalassemia were recruited. Analyses were performed for the specific analysis of the paternal mutation. All of the four commonest -thalassemic mutations, namely CD 41/42 (-CTTT), IVS2 654 (C>T), nt 28 (A>G) and CD 17 (A>T), found in Southeast Asians have been evaluated. The paternally-inherited fetal genotype was correctly identified in 20 of the 23 pregnancies (three false-negatives). This study thus demonstrates that fetal DNA can be robustly detected in maternal plasma by mass spectrometry, and paves the way for high-throughput non-invasive prenatal diagnosis.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 137 from 2003 ASHG Annual Meeting

Fetal DNA in Maternal Plasma Circulates as Apoptotic Bodies: Elucidation of the structural nature of fetal DNA for non-invasive prenatal genetic diagnosis. *F.Z. Bischoff*[1], *D. Dang*[1], *C. Horne*[2], *D. Marquez-Do*[1], *W.R. Brinkley*[3], *D.E. Lewis*[2], *J.L. Simpson*[1]. 1) Dept OB/GYN; 2) Dept Immunology; 3) Dept Cell Biology, Baylor Col Medicine, Houston, TX.

Cell-free fetal DNA is present in the maternal circulation and may be utilized for non-invasive prenatal genetic diagnosis. However, the biological (structural) nature of this DNA and the mechanisms underlying its variation in maternal plasma are unclear. Because great interest lies in its diagnostic utility, further molecular characterization for improved isolation and/or enrichment of fetal DNA is warranted. Because this DNA is stable following venupuncture and cleared rapidly after birth, we hypothesize that the majority of fetal DNA likely circulates in membrane bound vesicles (apoptotic bodies). DESIGN: Our goal was to determine the biological nature of circulating fetal DNA in maternal plasma using detection of fetal specific sequences in male pregnancies. METHODS: Following IRB approval and informed consent, plasma was separated by centrifugation (x800g) from 12-16 week gestation maternal blood (n=5) subsequently confirmed to have a male fetus. Acridine orange (AO), a nucleic acid stain, was used to label the recovered plasma, followed by flow cytometric separation of the AO positive staining (non-cellular) fraction. Sorted maternal plasma was then subjected to fluorescent microscope analysis as well as real-time PCR for FCY (fetal) and GAPDH (fetal and maternal) quantitative sequence detection. RESULTS: We demonstrate for the first time that apoptotic bodies exist in maternal plasma. Moreover, some of these bodies contain fetal sequences. Real-time PCR analysis of the sorted maternal plasma demonstrated significant enrichment with mean detection of 1 FCY to 29 GAPDH copies per reaction compared to non-sorted maternal plasma (ranging from1 FCY to 100-1000 GAPDH copies per reaction). CONCLUSION: Fetal DNA appears to circulate in apoptotic bodies, for in these fractions containing these bodies, FCY sequence is also present. Identifying the biological nature of circulating fetal nucleic acids will facilitate the molecular characterization of this DNA in both normal and abnormal pregnancies.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 138 from 2003 ASHG Annual Meeting

The gestational age-related increase in the frequency of hyperdiploid cytotrophoblasts at the human fetal-maternal interface as a possible mechanism to limit cell proliferation/invasion. *J.F. Weier[1, 2], C.K. Jung[3], Y. Zhou[3], H-U.G. Weier[2], L.W. Chu[2], A.A. Wright[3], S.J. Fisher[1,3,4]*. 1) Dept Ob/Gyn & RS,University of California, San Francisco,CA; 2) Life Sciences Division, E.O. Lawrence Berkeley National Laboratory, Berkeley, CA; 3) Dept Stomatology, UCSF, CA; 4) Dept Anatomy & Pharm Chem, UCSF, CA.

In most pregnancies, the fetus and the placenta have the same chromosomal complement because both lineages are descendants of the same zygote. But in 1-2% of viable pregnancies, chorionic villus sampling reveals a cytogenetic abnormality confined to the placenta. To date most cytogenetic studies have focused on cells cultured from floating villi. Thus, little is known about the karyotype of the cytotrophoblasts (CTBs) derived from anchoring villi that invade the uterine wall. Interestingly, the equivalent mouse trophoblast population undergoes sucessive rounds of DNA replication without cell division, which results in amplification of their entire genome. We applied multicolor fluorescence in situ hybridization to investigate the chromosomal composition of interphase cells from human floating or anchoring villi from first trimester to term. Isolated CTBs were initially analyzed using DNA probes for chromosomes X, Y, and 16. Male CTBs showed the presence of two X chromosomes in all the studied placentas. We then analyzed CTBs from 23 different placentas at different gestational age. The analysis revealed aneuploid cells that comprised from ~10%-60% of the population. We also observed a positive correlation between the rate of aneuploidy and increasing gestational age. Most abnormalities were a hyperdiploid karyotype with preferential gain of chromosome 16 and X. When scoring 12 different chromosomes in CTBs, the results showed a rate of aneusomy approaching 100%. Cells with tetraploidy or higher ploidy levels were sparse in our preparations suggesting that invasive CTBs at the uterine wall did not go through endoreduplication. We conclude that aneuploidy at the fetal-maternal interface is not an abnormality, but part of the normal placentation program which helps to limit the proliferative potential of these otherwise normal cells.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 139 from 2003 ASHG Annual Meeting

ANEUPLOIDY RESCUE AFTER FEMALE MEIOSIS I AND FOLLOW UP ANALYSIS OF ITS OUTCOME IN RESULTING PREIMPLANTATION EMBRYOS. *A. Kuliev, J. Cieslak, Z. Zlatopolsky, Y. Illkevitch, I. Kirilova, Y. Verlinsky.* Reproductive Genetics Institut, Chicago, Il.

We previously demonstrated that 43% of human oocytes obtained from women of advanced reproductive age are aneuploid after meiosis I. In addition, 37% of oocytes become aneuploid after meiosis II, of which 48% have also preceding meiosis I errors. The sequential meiosis I and meiosis II errors resulted in a balanced chromosomal status in 32.5% of these oocytes, representing a possible phenomenon of aneuploidy rescue described in post-zygotic embryo development. The proportions of oocytes with sequential meiosis I and meiosis II errors for specific chromosomes were 17.2%, 21.4%, 10.1%, 19.6% and 18.4% for chromosomes 13, 16, 18, 21, and 22, respectively, resulting in a balanced oocyte for these specific chromosomes in 4.8%, 5%, 4.1%, 15.6% and 7.8%, respectively. To investigate the post-zygotic outcome of these sequential meiosis I and meiosis II errors, 100 preimplantation embryos resulting from such balanced oocytes were followed up by FISH, with application of commercial probes specific for five chromosomes (chromosomes 13,16, 18, 21 and 22) (Vysis). Follow up analysis of 100 embryos deriving from the corresponding balanced oocytes (12 balanced for chromosomes 13, 17 balanced for chromosomes 16, 1 balanced for chromosomes 18, 20 balanced for chromosomes 21, 19 balanced for chromosomes 22 and 31 balanced for more than one chromosome) showed as high as 82% prevalence of chromosomal abnormalities in the resulting embryos, of which 48% were with mosaicism, involving the same or different chromosomes. Of only 18 (18%) chromosomally normal embryos obtained from the balanced oocytes, 8 originated from oocytes balanced chromosome 21, 5 from balanced 22, 3 from balanced 13, 1 from balanced 16, and 1 from balanced for more than one chromosome. The data suggest that the embryos originating from the oocytes with sequential meiosis I and meiosis II errors, resulting in a balanced chromosomal status, are not suitable for transfer, as chromosomal errors in meiosis may predispose to further chromosomal abnormalities at the cleavage stage.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Prenatal testing decision-assisting tool: Results of a randomized trial. *M. Kuppermann[1], M.E. Norton[1], E. Gates[1], L.A. Learman[1], V. Feldstein[1], J. Lewis[2], A.E. Washington[1], R.F. Nease[3].* 1) Dept Ob/Gyn,UCSF, San Francisco, CA; 2) Kaiser, San Francisco CA; 3) Express Scripts and Wash U, St Louis MO.

Prenatal testing has become increasingly complex. A broad array of options is available, while decisions regarding prenatal testing are value laden and should reflect informed patient preferences. We constructed and evaluated an interactive computerized d ecision-assisting tool (PT Tool) to help pregnant women decide which tests to undergo. PT Tool includes educational material, tailored risk estimates, values clarification and strategy building exercises. 496 pregnant women were randomized to PT Tool or a computerized version of California's educational pamphlet (control). Satisfaction, knowledge, risk comprehension, and decisional conflict were assessed at baseline, 1-2 weeks post program and at 30 weeks gestation. We also assessed inclinations toward h a ving screening (nuchal transluceny (NT) and serum screening) or diagnostic tests (chorionic villus sampling (CVS) and amniocentesis) immediately before and after, and 1-2 weeks after viewing their assigned program. At 30 weeks gestation, participants we re asked which prenatal tests they underwent. We hypothesized that PT Tool users would 1) be more knowledgeable about prenatal testing and their risk of a DS-affected fetus, 2) be more satisfied, 3) have less decisional conflict; that 4) PT Tool viewers < 35 would be more inclined than controls to undergo invasive testing, and 5) those >35 would be more inclined to undergo screening. Compared to controls, PT Tool users had greater knowledge (p<.0001), better estimated their risk of carrying a DS-affected fet us (p<.0001), were more satisfied (p<.0001), and had less decisional uncertainty (p=.006) and better perceived decision making effectiveness (p =.004). No differences were observed in inclinations toward invasive testing for younger women, but PT Tool vie wers >35 were more likely to undergo invasive testing (71 versus 56%, p=.047). PT Tool has a significant positive impact on knowledge, satisfaction and decisional conflict. It also increased utilization of invasive testing among women currently eligible for CVS and amniocentesis.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 141 from 2003 ASHG Annual Meeting

Amniocentesis uptake after maternal serum screen positive for Down syndrome varies with patient demographics but not with numeric risk.

K. Borsack[1,2], N. Nakata[2], A. Metzenberg[1], S. Morton[3], J. Greenberg[3], J. Siegel-Bartelt[3]. 1) Genetics, California State, Northridge, Northridge, CA; 2) Genzyme Genetics; 3) Southern California Kaiser.

We retrospectively studied 5979 women who were maternal serum screen positive for Down syndrome(MSS+DS) seen by Genzyme Genetics in southern California from the years 1999, 2000 and 2001. We compared amniocentesis uptake to previously coded demographic factors and numeric risk for Down s. **Overall amniocentesis uptake was 51.2%**;. The cut-off risk for referral for MSS+DS in California is a numeric risk for Down syndrome of 1/190 or greater. **Amniocentesis uptake did not vary significantly by numeric risk level for Down s.** However, women 37+ years had higher amniocentesis acceptance if their risk increased compared to *a priori* age related risk 46.6%[544/1168] versus women 37+ whose risk decreased 37.8%[223/590]. **Highly significant (p<0.001) demographic factors included**: 1) **maternal age**, 29-36 years had the highest uptake 59.7% [1642/2749] compared to <29years 48.9% [650/1327] and >36 years was 42.3%[593/1403]: 2) one or more **previous elective abortion** 62.6%[584/932]: 3) **no previous spontaneous losses** 53.2%[2282/4291] compared with 1 previous SA 47.9%[485/1012], 2 SAs 41.5% [134/323] and 3+SAs 45.3%[58/128]. **Low parity** was associated with increased uptake but was not independent of maternal age. Primiparous women had amniocentesis uptake of 54.1%[952/1761]; P1 had uptake of 57%[1037/1815]; P2--48.5%[531/1095]; P3--44.4%[264/595]; P4--39% [99/254] and P5+--32.5%[76/234]. **Ethnic group differences in amniocentesis uptake were highly significant**: Caucasian 56.3%[819/1430]; African American 58.5%[266/455]; Asian 63.7%[559/878]; Ashkenazi Jewish 75.3% [125/166]; Middle Eastern 64.3%[72/112]; Hispanic choosing counseling in English 50.3%[660/1311]; Hispanic with counseling in Spanish 34.6%[554/1599]. We conclude that demographic and acculturation factors are strongly associated with patient decisions and should be considered in public health care planning. Patients most likely to accept amniocentesis are non-Hispanic women with 1 child or no children, without previous miscarriage, ages 29-36 years.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 142 from 2003 ASHG Annual Meeting

Choroid plexus cysts are not associated with significant cognitive, motor, language or adaptive behaviour delays in early childhood. *F.P. Bernier[1], S. Crawford[2], D. Dewey[2,3]*. 1) Dept of Medical Genetics, University of Calgary, Alberta, Canada; 2) Behavioral Research Unit, Alberta Children's Hosp, Calgary, AB, Canada; 3) Dept of Pediatrics, University of Calgary, Alberta, Canada.

The choroid plexus is easily identified within the fetal cranium due to its echogenicity and choroid plexus cysts (CPC) are associated with an increased risk of aneuploidy, however the exact magnitude of this increase remains disputed. When no chromosome abnormality is detected, CPCs are considered to be benign variants of choroid development although we found no substantive evidence in the literature to confirm that CPCs are not associated with developmental delays. The purpose of this retrospective double cohort study was to evaluate development in children who had CPCs detected prenatally and to determine whether their presence during the second trimester was associated with significant cognitive, motor, language or adaptive behavior delays. Our study cohort (N=38) had a mean age of 2.85 (sd 0.84) years and the control cohort (N=63) had a mean age of 4.92 (sd 1.56) years. Although the age difference between our cohorts was significant, there were no differences in socioeconomic status, sex, birth weight or gestational age. Cognitive data showed no significant difference in Full Scale IQ using the WISC III or WIPPSI-R (CPC = 113.11, control = 115.85). Scores on standardized measures of language, motor and adaptive functioning also did not show any significant group differences. Overall, we conclude that the presence of isolated CPC's on midtrimester ultrasound is not associated with any significicant neurocognitive delays in early childhood. Families should therefore continue to be reassured that in the absence of fetal aneuploidy, CPC's are benign anatomical variants.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Low unconjugated estriol pregnancies and prenatal diagnosis. *K. Pilchman, S. Mueller, P. Pearle, R. Ruzika, K. Reddy.* Genzyme Genetics, Orange, CA.

Low unconjugated estriol (uE3) in 2nd trimester maternal serum triple marker screen may serve as an indicator for fetal X-linked steroid sulfatase deficiency (STS) (MIM # 308100; affecting 1/2,000 males), trisomy 18, triploidy or Smith-Lemli-Opitz syndrome (SLOS)(MIM # 270400; affecting 1/20,000 Caucasians).A total of 85 patients with low uE3 had genetic testing. Median maternal age (MA) was 28.6 years (range 16 - 41). Average gestational age (GA) was 18.4 weeks (range 13.9 - 20.9). Of 30 fetuses with an abnormal ultrasound (US) 10 had abnormal karyotypes and 1 had STS deletion. SLOS: 77 pregnancies with an average uE3 of 0.25 MoM and GA adjusted risk of 1/10 to 1/49 (cutoff 1/50) were tested. One fetus [1/77 (1.3%)] had elevated 7-dehydrocholesterol in the SLOS range [4,641 ng/ml (normal 6 ng/ml SD3)]. US showed a prominent nuchal fold of 4.4 mm and questionable female genitalia, while the karyotype was 46,XY. Our incidence of 1.3% fetuses with SLOS is in the expected range of 1-2/100. US of 3 fetuses showed anencephaly, all had mild increase in 7-dehydrocholesterol (14-28 ng/ml). STS: 24 pregnancies with average uE3 of 0.19 MoM had a STS deletion in 11/21 (52%) fetuses (3 mothers confirmed to be carriers) and in 3/3 (100%) pregnant women who declined fetal testing. One fetus had an interstitial deletion of STS and KAL (Kallman syndrome) loci but the Xp subtelomere was present. In the absence of strong family history of ichthyosis, mothers of male fetuses with STS deletion need to be tested. Also, a larger deletion may be ruled out using KAL and subtelomere Xp/Yp FISH probes. Our case with an interstitial deletion raises the concern that the NLGN4 gene for autism/Asperger syndrome (MIM # 300427), telomeric to STS, may be deleted. Hence a probe set that includes NLGN4 is important, to assess larger deletions and to counsel about the mental well being of the fetus.An abnormal karyotype was found in 14/85 [16%] fetuses. The abnormalities were: trisomy 18 [6/14 (43%) with risk of 1/10-22 based on triple marker screen and MA]; triploidy [5/14 (36%). 69,XXX (3), 69,XXY(1), & 68,XX(1)] and sex chromosome abnormalities [3/14 (21%). 46,X, i(X)(q10), mos 45,X/46,i(X)(q10), & mos 46,X,inv(Y)(p11.2q11.2)[12]/ 47,X,inv(Y)(p11.2q11.2)+16[5]].

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 144 from 2003 ASHG Annual Meeting

Registry of Pregnancies Exposed to Chemotherapeutic Agents. *S. Hassed, C. Mumm, A. Reed, R. Kohl, J. Mulvihill.* Section of Genetics, Department of Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Background: To clarify the effects of cancer chemotherapy on the developing fetus and to provide an international resource for clinical counselling, a computerized registry of pregnancies exposed to chemotherapy agents was developed in 1984. The registry was developed at the National Cancer Institute and is maintained at The University of Oklahoma in a Microsoft Access database. Currently, the registry includes 361 cases.
Methods:: The cases were collected from western medical literature since 1952, physician referrals, clinical records, family study and self-referral. Information was collected on medical and obstetric history, drug and radiation exposures, birth outcome and a weekly chronicle of the pregnancy.
Results: Of the 361 cases, 293 reported no structural anomolies and the remaining 68 cases reported a total of 181 defects. The anomaly rate was 19%. Exposure begun in the 1st trimester had the most profound effects on the fetus with an anomaly rate of 27%. The anomaly rate with pre-conception exposure was 25%. However, the majority of these cases (91%) continued therapy into pregnancy. Exposure begun in the 2nd and 3rd trimesters produced an anomaly rate of 10%. The fetal outcomes were 310 live births, 27 elective abortions, 15 spontaneous abortions, 5 stillborn and 4 others. Most of the patients had exposure to more than one chemotherapeutic agent during their pregnancy.
Conclusions: As expected, trimester of exposure to chemotherapy agents is an important determinant of the anomaly rate. With delayed pregnancies, the occurrence of malignancies during pregnancy is likely to increase, highlighting the specific need for a registry to provide rapid access to relevant cases in the literature to aid in counselling and medical decision-making.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Identification of a New Disease Gene Causing Lafora Progressive Myoclonus Epilepsy. *E.M. Chan[1], E.J. Young[1], L. Ianzano[1], I. Munteanu-Oprea[1], X. Zhao[1], C.C. Christopoulos[1], G. Avanzini[2], M. Elia[3], C.A. Ackerley[1], N.J. Jovic[4], S. Bohlega[5], E. Andermann[6], G.A. Rouleau[7], A.V. Delgado-Escueta[8], B.A. Minassian[1], S.W. Scherer[1].* 1) Research Institute, The Hospital for Sick Children, Toronto, Canada; 2) Instituto Nazionale Neurologico, Milan, Italy; 3) Oasi Institute for Research on Mental Retardation and Brain Aging, Enna, Italy; 4) Clinic of Neurology and Psychiatry for Children and Youth, Belgrade, Serbia & Montenegro; 5) King Faisal Specialist Hospital, Riyadh, Saudi Arabia; 6) Montreal Neurological Institute, McGill University, Quebec, Canada; 7) McGill University Health Centre Research Institute, Montreal General Hospital, Quebec, Canada; 8) Brain Research Institute, University of California Los Angeles, USA.

Lafora disease (LD) is the most common and severe form of adolescent progressive epilepsy with increasing seizures, dementia, and death usually within 10 years of onset. At the cellular level, LD is characterized by an endoplasmic reticulum (ER)-associated accumulation of starch-like glucose polymers called polyglucosans (or Lafora bodies). Inheritance is autosomal recessive with genetic heterogeneity but the clinical presentation is homogeneous. We previously discovered that mutations in the gene *EPM2A* on chromosome 6q24 encoding a dual-specificity phosphatase (named laforin) with a carbohydrate binding domain, cause LD in a subset of patients (*Nature Genetics*, 1998). To identify additional genes involved in LD, we performed linkage analysis in families that do not segregate *EPM2A* mutations and, ultimately, identified a second LD locus on chromosome 6p22.3 (*Journ. of Medical Genetics*, In press). Here, we describe the positional cloning of a novel gene named, *EPM2B*, at the 6p22.3 locus and show that it causes LD. Seventeen distinct DNA sequence variations in *EPM2B* predicted to cause deleterious effects on the protein product, were found to co-segregate with LD in 26 families (*Nature Genetics*, submitted). In total, 88% of our LD family collection can now be accounted for by mutations in *EPM2A* (48%) and *EPM2B* (40%). We have also completed cell localization studies and show the *EPM2B* product, malin, localizes to the ER where Lafora bodies are found.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 146 from 2003 ASHG Annual Meeting

Identification of the Cayman Ataxia gene via the mouse mutant jittery: A novel neuron-specific CRAL-TRIO domain protein. *M. Burmeister*[1, 2, 3], *J.M. Bomar*[1], *P.J. Benke*[5], *E. Slattery*[1], *R. Puttagunta*[1], *L. Taylor*[1], *E. Seong*[1], *A. Nystuen*[6], *W. Chen*[7], *R.L. Albin*[4], *P.D. Patel*[1, 2], *R.A. Kittles*[7], *V.C. Sheffield*[6]. 1) Mental Health Research Institute, University of Michigan, Ann Arbor; 2) Dept. of Psychiatry, Univ. of Mich; 3) Dept. of Human Genetics, Univ. of Mich; 4) Dept. of Neurology, Univ. of Mich; 5) Dept. of Pediatrics, Univ. of Miami, FL; 6) Dept.s of Pediatrics, Human Genetics and Howard Hughes Medical Institute, Univ. of Iowa, Iowa City; 7) National Human Genome Center at Howard Univ., Washington, DC.

Cayman ataxia (*ATCAY*) is a recessive cerebellar ataxia restricted to one area of Grand Cayman Island where founder effect and inbreeding has led to a high frequency. Comparative mapping suggested that the *ATCAY* gene on 19p13.3 may be homologous to the recessive ataxic mouse mutant jittery on mouse Chromosome 10. Screening genes in the overlapping critical region, we identified mutations in a novel predicted gene in three mouse jittery alleles, including the first mouse mutation caused by an Alu-related (B1 element) insertion. Two mutations, a missense and a predicted splice mutation, were found exclusively in all Cayman Ataxia patients but not in over 1000 control chromosomes. The *ATCAY/Atcay* gene encodes a neuron-restricted protein, CAYTAXIN. CAYTAXIN contains a CRAL-TRIO motif common to proteins that bind small partially hydrophobic molecules. Mutations in another CRAL-TRIO domain-containing protein, tocopherol-transferase, cause Vitamin-E responsive ataxia. 3D protein structural modelling predicts that the CAYTAXIN ligand is more polar than vitamin E. Identification of the CAYTAXIN ligand may thus help identify a therapy for this severe ataxia.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 147 from 2003 ASHG Annual Meeting

Neurological mouse models for Friedreich ataxia. *D. SIMON[1], H. SEZNEC[1], L. REUTENAUER[1], A. GANSMULLER[1], P. RUSTIN[2], M. KOENIG[1], H. PUCCIO[1].* 1) IGBMC, Illkirch, FRANCE; 2) HOPITAL NECKER, Paris, FRANCE.

Friedreich ataxia (FRDA), the most common autosomal recessive ataxia, is characterized by degeneration of the large sensory neurons of the spinal cord and by cardiomyopathy. It is caused by severely reduced levels of frataxin, a mitochondrial protein involved in iron-sulfur cluster biosynthesis. Our initial attempts to create a neurological mouse model for FRDA resulted in mutant animals with a multisystemic disease and a short life span.We have now inactivated the frataxin gene in a spatio-temporal manner by a tamoxifen inducible recombinase under the control of the neuron-specific Prion protein promoter. We obtained two lines which develop progressive neurological symptoms resembling FRDA. Rotarod and footprint studies revealed a progressive motor incoordination beginning at 10 weeks and a clear ataxia leading to loss of spontaneous ambulation at 1 year, without muscle strength reduction. Furthermore, electromyographic studies revealed a selective deficiency of the proprioceptive sensory neurons with normal motor nerve conduction, corresponding to hallmark features of FRDA. Histological studies showed both spinal cord and dorsal root ganglia (DRG) anomalies but absence of peripheral neuropathy. In addition, one line revealed a cerebellar granule cells loss, while both lines had Purkinje cell arborisation defects. These lines represent the first FRDA models with a slowly progressive neurological degeneration. The newly identified pathological mechanism in the DRG is a autophagic process, leading to removal of mitochondrial debris and lipofuscin deposits, most likely corresponding to oxidized catabolic products. These models therefore represent excellent models for FRDA to unravel the pathological cascade, and to test compounds that interfere with the degenerative process, such as anti-oxidants, currently the best pharmacological candidates.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 148 from 2003 ASHG Annual Meeting

NIPA1 gene mutations cause autosomal dominant hereditary spastic paraplegia (SPG6). *S. Rainier*[1], *J-H. Chai*[3], *D. Tokarz*[1], *R.D. Nicholls*[3], *J.K. Fink*[1,2]. 1) Dept Neurology, 5110 CCGC, Univ. of Michigan, Ann Arbor, MI; 2) The Geriatric Research, Education, and Clinical Center, Ann Arbor Veteran's Affairs Medical Center, Ann Arbor, MI; 3) Dept of Psychiatry, Univ. of Pennsylvania, Philadelphia, PA.

The hereditary spastic paraplegias (HSPs) are genetically heterogeneous disorders characterized by progressive lower extremity spastic weakness. Autosomal dominant HSP (ADHSP) linked to chromosome 15q (SPG6; Fink et al AJHG 1995;56:188-192) begins in the second to fourth decade and progresses inexorably. The SPG6 locus involves the proximal 15q region often deleted in Prader-Willi and Angelman syndromes (PWS and AS). PWS and AS are imprinted disorders. We studied a large SPG6 kindred and found no evidence of genetic imprinting. Therefore, we analyzed as SPG6 candidates the four unique, non-imprinted genes mapped proximal of the 15q imprinted domain (Chai et al., AJHG, in press). A nucleotide substitution (nt 159 C>G) in NIPA1 resulted in an amino acid substitution (T45R) in each affected subject of the SPG6 family, but was not present in unaffected subjects of this family nor in 115 control subjects. This amino acid is conserved in mouse, chicken and fish (ibid). We analyzed 62 additional ADHSP kindreds and discovered that affected subjects in one unrelated kindred had precisely the same NIPA1 mutation. The two ADHSP families are of Irish and Iraqi ancestry, and are unrelated based on haplotype analysis, suggesting that the identical NIPA1 mutation arose independently. Our observations of the same NIPA1 gene mutation in two unrelated ADHSP kindreds that disrupts an inter-species conserved amino acid and that was absent in control subjects argue strongly for the pathogenic significance of this mutation. Since NIPA1 haploinsufficiency (as may occur in PWS and AS) does not cause spastic paraplegia, the NIPA1 mutation is likely pathogenic through a dominant negative mechanism. NIPA1 is highly expressed in neuronal tissues and encodes a putative membrane spanning protein that may function as a membrane transporter or receptor. These findings advance our knowledge of the basis of HSP and related neurologic disorders.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 149 from 2003 ASHG Annual Meeting

Deletion of murine Smn exon 7 directed to liver leads to embryonic lethality: further evidence for a dosage effect of SMN in Spinal Muscular Atrophy (SMA). *J. Vitte[1], B. Davoult[2], M. Mayer[3], N. Roblot[1], V. Joshi[1], J. Vadrot[2], F. Tronche[4], F. Kemeny[2], J. Melki[1].* 1) Molecular Neurogenetics Laboratory, INSERM E-223, Evry, France; 2) Service d'Anatomie Pathologique, Centre Hospitalier Sud-Francilien, Evry; 3) Hopital Saint-Vincent de Paul, Paris; 4) College de France, Paris.

SMA is a recessive autosomal disorder characterized by degeneration of lower motor neurons and caused by mutations of the SMN1 gene. Whether SMN has an essential role in all mammalian cell types or a specific function in the neuromuscular system was unknown. In order to answer this question, deletion of murine Smn exon 7, the most frequent mutation found in human SMA, has been directed to liver, a tissue non-affected in human SMA. Alfp-Cre transgene (Alfp-cre) that expresses the Cre recombinase in liver only from embryonic day 10 was used to target the mutation from Smn exon 7 allele flanked by two loxP sites (SmnF7). No (Alfp-Cre, SmnF7/F7) living mutant mice was detected out of 200 newborns. Lack of mutant embryos was observed from 18 d.p.c. indicating that homozygous deletion of Smn exon 7 directed to liver results in late embryonic lethality. Examination of mutant embryos showed dramatic liver atrophy associated with rare hepatocytes and dramatic reduction of -fetoprotein level when compared to control embryos. In heterozygous mice, no liver change was noticed indicating that half dose of SMN is sufficient for normal liver function and excludes a dominant negative effect of Smn exon 7 deletion in vivo. In human, SMN1 is duplicated in a highly homologous copy SMN2. The residual amount of SMN produced by the SMN2 gene, which remains present in patients, is likely sufficient to ensure normal functions of various organs including liver but not motor neurons. Consistently, our data showed normal liver function in childhood SMA patients. Our results suggest that SMN, a protein involved in RNA metabolism, plays a similar function in all cells. Identifying RNA encoding proteins implicated in the structural specificities of motor neurons, one of the largest cells in the body, should contribute to elucidate SMA pathogenesis.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Disruption of the endocytic protein HIP1 results in major neurological deficits and decreased AMPA receptor trafficking. *M.M. Metzler[1], L. Gan[1], B. Li[2], J. Georgiou[3], C.-A. Gutekunst[4], Y. Wang[5], E. Torre[4], Y.T. Wang[5], J.C. Roder[3], L.A. Raymond[2], M.R. Hayden[1]*. 1) Dept Medical Genetics, CMMT, UBC, Vancouver, BC, Canada; 2) Kinsmen Laboratory, Dept of Psychiatry, UBC, Vancouver, BC, Canada; 3) Samuel Lunenfeld Res Inst, Mount Sinai Hospital, Toronto, ON, Canada; 4) Emory Univ School of Med, Atlanta, GA, USA; 5) Dept of Medicine and The Brain Res Centre, UBC, Vancouver, BC, Canada.

Huntingtin interacting protein 1 (HIP1) is a recently identified component of clathrin-coated vesicles that plays a role in clathrin-mediated endocytosis. The initial identification of HIP1 resulted from its interaction with the polyglutamine-containing protein huntingtin that, in its polyglutamine-expanded form, causes Huntington's Disease (HD). To explore the normal function of HIP1 *in vivo*, we have created mice with a targeted mutation in the HIP1 gene (HIP1-/-). HIP1-/- mice develop a neurological phenotype by 3 months of age manifest with a failure to thrive, tremor and a gait ataxia secondary to a rigid thoracolumbar kyphosis. These neurological signs are accompanied by decreased assembly of endocytic protein complexes on liposomal membranes. In primary hippocampal neurons, HIP1 colocalizes with GluR1-containing AMPA receptors and becomes concentrated in cell bodies following AMPA stimulation. Moreover, a profound dose-dependent defect in clathrin-mediated internalization of GluR1-containing AMPA receptors was observed in neurons from HIP1-/- mice. Together, these data provide strong evidence that HIP1 regulates AMPA receptor trafficking in the CNS through its function in clathrin-mediated endocytosis.

Supported by Canadian Disease Genetics Network, Canadian Institutes of Health Research and Merck-Frosst Canada.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

The Rho-GEF Pebble is a target of the UBE3A ubiquitin ligase responsible for Angelman syndrome. *L. Reiter*[1], *M. Bowers*[1], *T. Seagroves*[1], *J.-H. Jiang*[2], *A. Beaudet*[2], *E. Bier*[1]. 1) Department of Biology, University of California, San Diego, La Jolla, CA; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Angelman syndrome (AS) results from the inheritance of a maternal deficiency of the *UBE3A* gene, which encodes an E3 ubiquitin ligase. The mouse model of AS suggests that the learning and motor control defects associated with AS result from abnormally elevated levels of a set of proteins specifically targeted for degradation by UBE3A. Although a small number of these protein targets are known, the identity of the key effectors responsible for the neuronal defects observed in AS remain elusive. We chose to identify the targets of UBE3A using the genetic model organism *Drosophila melanogaster*. We identified a clear *Drosophila* homolog (*das*) of human UBE3A using the Homophila database. Mis-expression of both human and fly versions of this ubiquitinase resulted in the identification of 19 putative target proteins that were down-regulated by either human or fly ubiquitinase. One of these proteins is the Rho-GEF *pbl*, which is involved in cytokenesis as well as neuronal path finding in *Drosophila*. The highly conserved mammalian counterpart of *pbl* is the proto-oncogene *Ect-2*. We find that Pbl/Ect-2 protein levels can be regulated by UBE3A expression in both fly and mouse model systems. This orthologous posttranslational pathway of regulation is essential to neuronal outgrowths in both organisms and should be conserved in humans as well. Pbl is, therefore, the most clinically relevant target of UBE3A found to date and its mis-regulation may underlie the learning and motor defects observed in AS patients.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Mutations in a novel gene cause hereditary sensory and autonomic neuropathy type II. *M. Samuels[1], R. Lafreniere[2], M. MacDonald[1], J. MacFarlane[1], M.-P. Dube[1], M. O'Driscoll[3], S. Meilleur[2], J. Thompson[1], Y.P. Goldberg[1], B. Brais[4], W. Pryse-Phillips[3], R. Green[3], B. Younghusband[3], M. Hayden[1,5], R. Sherrington[1], G. Rouleau[2,6]*.
1) Xenon Genetics, Inc, Burnaby, BC, Canada; 2) Xenon Genetics Research, Inc, Montreal, QC, Canada; 3) Discipline of Genetics, Faculty of Medicine, Memorial Univ of Newfoundland, St. John's, NF, Canada; 4) Dept de Medecine de l'Univ de Montreal, Montreal, QC, Canada; 5) Dept of Medical Genetics, Univ of British Columbia, Vancouver, BC, Canada; 6) Centre for Research in Neuroscience, McGill Univ, Montreal, QC, Canada.

Hereditary sensory and autonomic neuropathy type II (HSAN II, OMIM 201300) is an autosomal recessive disorder characterized by absence of pain, temperature and touch sensations due to reduction or absence of myelinated peripheral sensory neurons. We identifed two large pedigrees segregating the disorder (first published in 1946) in an isolated population living in Newfoundland, and performed a 5 cM genome scan on 22 family members using 811 markers. Linkage and haplotype analysis in these families, and in two additional patients of French-Canadian origin, identified a unique chromosomal locus with a maximum LOD score of 8.4, defining a minimal candidate interval of 1.06 Mbp containing 8 known genes. Sequencing of the coding regions of these genes did not reveal a causal mutation, however 3 different loss-of-function mutations were detected in affected individuals in a novel gene identified within the chromosomal interval. None of these mutations was observed in a set of 96 control individuals. This novel gene (HSN2) is located within the intron of another gene, appears to contain a single exon, and is highly conserved in vertebrates (87% identity between human and mouse). Based on RT-PCR using mouse and human tissues, the HSN2 gene may be expressed at low level in dorsal root ganglion, and is undetectable in other tissues. HSN2 could function in the development or maintenance of peripheral sensory neurons or their supporting Schwann cells. The protein contains a putative signal peptide and may encode a novel nerve growth factor.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 153 from 2003 ASHG Annual Meeting

ITSN1 is a critical gene for early development of neuroblast migration demonstrated by human monosomy 21 and Itsn1 deficiency mouse. *X.N. Chen[1], Y.W. Brown[1], L. Flores-Sarnat[2], H.B. Sarnat[2], J.R. Korenberg[1].* 1) Med Genet, Cedars-Sinai Medical Center, Los Angeles, CA; 2) Peds Neuro/Neuropath, Cedars-Sinai Medical Center, Los Angeles, CA.

Neuroblast migration is a complex process that requires the coordinated functions of multiple genes. Human defects in this process cause cortical dysgenesis (CD) and lead to profound mental retardation (MR) and epilepsy. As reported previously, we identified a human CD region (2-3Mb on Chr 21q22) in patients with partial monosomy 21. This region contains Intersectin 1 (ITSN1), a binding partner of dynamin that is involved in clathrin-mediated endocytosis and expressed in human and mouse neurons by using our ITSN1 antibody. As a candidate for human CD, we generated a K/O mouse for ITSN1 for which our initial neuroanatomic analysis of homozygotes and heterozygotes revealed gross abnormalities including a globally thinner cerebral cortex, absence of corpus callosum (CC), and dysgenesis of hippocampus and cerebellum, all of which are similar to human del 21 phenotypes. We now report findings on 7 Itsn1$^{-/-}$adult mouse brains that unexpectedly show disturbed neuronal lamination and broadening in all CA regions of the hippocampus and discontinuities of the pyramidal cell layers in CA3 that reminiscent to findings seen in the Lis1 and doublecortin (Dcx) deficient mouse models. In addition, we found poorly defined cortical plates in the Itsn1$^{-/-}$embryonic days 13 and 15. These findings strongly suggest that ITSN1 might participate with LIS1 and DCX in a common pathway for cortical lamination in humans and mice. However, the absence of the CC uniquely seen in the ITSN1 K/O mouse as well as in Chr 21 del, suggests that ITSN1 does not share a simple biochemical pathway with LIS1 and DCX, but involves clathrin-mediated endocytosis. Our data show for the first time that 1) ITSN1 is necessary for the formation of midline brain structure including CC. 2) In addition to LIS1, DCX, Reln, and Cdk5, ITSN1 is necessary for the early development of neuroblast migration in both human and mouse. Its decreased expression may be largely responsible for the CD seen in del 21 and may contribute to the MR seen in Down Syndrome.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

D4Z4 hypomethylation in FSHD causes the transcriptional upregulation of 4qter genes. *S. van der Maarel[1], P. van Overveld[1], R. Lemmers[1], L. Sandkuijl[1], L. Enthoven[2], S. Winokur[3], G-J. van Ommen[1], G. Padberg[4], R. Frants[1]*. 1) Department of Human Genetics, Leiden Univ Medical Center, Leiden, Netherlands; 2) Division of Medical Pharmacology, Leiden/Amsterdam Center for Drug Research, Leiden University Medical Center, Leiden, The Netherlands; 3) Department of Biological Chemistry, College of Medicine, University of California, Irvine CA, USA; 4) Department of Neurology, University Medical Center Nijmegen, Nijmegen, The Netherlands.

The autosomal dominant myopathy facioscapulohumeral muscular dystrophy (FSHD) progressively and variably affects the muscles of the face, shoulder and upper arm. FSHD is caused by contraction of the polymorphic low copy D4Z4 repeat array on 4qter. Several observations suggest an epigenetic etiology in FSHD. First, the subtelomere of chromosome 10q harbors a highly homologous polymorphic repeat and size reductions of this repeat are non-pathogenic. Second, (partial) exchanges between the homologous repeat arrays on 4q and 10q are frequently observed but these exchanged repeats are only pathogenic when the contracted form resides on chromosome 4. Finally, contractions of the D4Z4 repeat array on 4qter per se are not sufficient to cause disease since pathogenic arrays are only associated with one of two allelic variants (4qA) of 4qter located distal to D4Z4. To obtain evidence for an epigenetic mechanism underlying FSHD pathogenesis, we studied the DNA methylation of D4Z4. We demonstrate that contraction of D4Z4 in FSHD patients causes hypomethylation of D4Z4. Providing biological significance for this finding, we show that in cultured cells, forced hypomethylation of D4Z4 results in transcriptional upregulation of 4qter genes at distance of D4Z4, similar to the inappropriate gene expression observed in FSHD muscle. Finally, also FSHD phenocopies, patients clinically identical to FSHD but without contraction of D4Z4, turn out to be hypomethylated at D4Z4. Together, these results strongly suggest that the cascade of epigenetic events causing FSHD involves hypomethylation of D4Z4, which in turn results in transcriptional upregulation of 4qter genes.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 155 from 2003 ASHG Annual Meeting

Solving the multipoint likelihood problem using haploid genotyping and likelihood factorization. *J.R. O'Connell.* University of Maryland, Baltimore, MD.

Computing multipoint likelihoods on pedigrees is one of the hardest problems in computational genetics. Despite significant algorithmic improvements over the past 20 years, exact calculations on large pedigrees with missing information and loops often remain intractable. In addition, dense marker maps of SNPs present new challenges to our algorithms that assume linkage equilibrium. We present a merger of molecular and mathematical techniques that solves the multipoint likelihood problem on a large class of these currently intractable complex pedigrees. We solve the problem by eliminating the computational cost of missing haplotype phase using molecular techniques and the computational cost of peeling individuals with missing genotype data using mathematical techniques. The molecular technique is conversion technology that uses mouse-human hybrids to isolate human haploid chromosomes and has been used to find mutations previously undetected using diploid data by eliminating the noise from the second chromosome. An individual in the haploid state has at most 2 phases compared to as many as 2 to the number of markers in the diploid state. The mathematical technique is a factorization algorithm that decomposes the pedigree into components with marker data and missing data, and computes the likelihood by summing over the haplotypes of the individuals connecting the components. Although the missing data component is intractable using current peeling algorithms, the likelihood is only a function of the founder source of the allele in each connecting individual, and thus can be efficiently calculated using graph theory methods. To illustrate our method we start with a fully typed CEPH pedigree with 6 grandchildren and then add 34 individuals with no data to connect the 4 grandparents as fourth cousins. The modified pedigree with 3 loops is intractable using diploid data beyond a few markers. However, assuming that the grandparents have haploid data, our factorization algorithm computes the exact likelihood with 50 markers in under a minute. We also present examples illustrating the potential of haploid data to completely transform family-based error checking, haplotyping, and linkage disequilibrium mapping in the study of complex diseases.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Computing pairwise single locus IBD probabilities in arbitrary pedigrees. *M. Abney.* Dept Human Genetics, Univ Chicago, Chicago, IL.

Computing probabilities of identity by descent (IBD) states for pairs of individuals at a single genotyped marker is a necessary step for some nonparametric linkage and multipoint IBD estimation methods. Many current techniques for calculating these probabilities are limited by either the size or complexity of the pedigree or the degree of missing genotype data. Here, we present a recursive method for computing all nine single locus IBD state probabilities for a pair of individuals, given their genotype at the locus and all ancestral genotype data, that can be used on pedigrees of arbitrary size and complexity. The method is equivalent to exact methods, which condition on all genotype data rather than just ancestral data, if there are no missing genotypes, and remains highly accurate and fast even with significant missing data. We compare the method to an exact algorithm for a variety of small pedigrees with and without missing genotypes. We also use the algorithm to compute the IBD probabilities at a genotyped marker for all pairs from both a simulated data set with a sample of 48 people from a pedigree of 160 individuals and from a real data set comprising a sample of 500 individuals drawn from a 13 generation, 1,623 person Hutterite pedigree.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 157 from 2003 ASHG Annual Meeting

Traits, traces and transposes: the Expectation Maximization (EM) algorithm in Quantitative Trait Locus (QTL) factor analysis. *L. Bauman*[1], *J.S. Sinsheimer*[1,2], *K. Lange*[1,2]. 1) Dept Biomathematics, UCLA Sch Medicine, Los Angeles, CA; 2) Dept Hum Gen, UCLA Sch Medicine, Los Angeles, CA.

The application of factor analysis to human genetics has the potential for the discovery of the coordinated control of multiple traits by common environment, common polygenes, or a single major gene. Classical factor analysis explains the covariation among the components of a random vector by approximating the vector by a linear transformation of a small number of uncorrelated factors. Although factor analysis seamlessly dovetails with the classical variance decompositions of biometrical genetics, estimation of the degenerate covariance matrices encountered in multivariate QTL mapping is difficult. We present the highlights of a cyclic EM algorithm that overcomes these difficulties by employing partial Cholesky decompositions and novel expansions of the traces of Kronecker products. We then compare the performance of the cyclic EM algorithm with the quasi-Newton approach currently implemented in the variance component software package FISHER.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Association Tests for Quantitative Traits in General Pedigrees. *E. Sobel*[1], *K. Lange*[1,2,3]. 1) Departments of Human Genetics; 2) Biomathematics and; 3) Statistics, UCLA, Los Angeles, CA.

Quantitative traits are inherently more informative than disease dichotomies. Many association tests for quantitative traits rely on statistical procedures such as analysis of variance that neglect familial correlations. However, with pedigree data, a better approach to association testing is to use variance component ideas and thus impose genotype or allele specific effects on trait means. This "measured genotype" approach controls for polygenic background while remaining in the domain of maximum likelihood estimation and likelihood ratio tests. Three objections can be made to this strategy. First, pedigree data may be ascertained and not random. Second, it is unclear how to handle missing data. Third, likelihood ratio distributions may be sensitive to both small sample sizes and departures from normality. We address all three concerns.

The first objection can be partially overcome by conditioning the observations on the trait values of probands. The second objection could be handled by using only individuals possessing both traits and marker genotypes. However, some of the most informative markers will be constructed by combining intragenic SNPs, in effect creating non-codominate markers. When marker genotypes are unobserved, or non-codominance partially obscures genotypes, we propose substituting conditional probabilities of genotypes for genotypes. This necessitates computing the expected number of marker alleles of each type harbored by each person. We demonstrate efficient computation of these expectations using all observed data, by Mendel (for smaller pedigrees) and SimWalk2 (for larger pedigrees). The third objection to the measured genotype strategy can be overcome by permutation tests. These tests rely on exchangeable groups of people such as sibships or spouse pairs. Each such group we call a permutation unit. Under the null hypothesis of no trait-genotype association, every permutation of trait values within a unit is equally likely. Using these procedures, we demonstrate a likelihood ratio test that accommodates multivariate traits, probands, polygenic background, missing traits, and non-codominant alleles.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 159 from 2003 ASHG Annual Meeting

Graphical modeling of the joint distribution of alleles at associated loci. *A. Thomas, N. Camp.* Genetic Epidemiology, 391 Chipeta Way Suite D, Salt Lake City, UT 84108.

Pairwise linkage disequilibrium, haplotype blocks and hot spots provide only a partial description of the patterns of dependences and independences between the allelic states at proximal loci. On the gross scale, where recombination and spatial relationships dominate, the associations can be reasonably described in these terms. However, on the fine scale of current polymorphism maps the mutation process is important and creates associations which are independent of the physical ordering and which can not be summarized with pairwise measures of association. Graphical modeling provides a standard statistical framework for characterizing precisely this sort of complex stochastic data. While graphical models are often used in situations where assumptions lead naturally to specific models, it is less well known that estimation of graphical models is also a well developed field. In this presentation decomposable graphical models are fitted to data from 25 SNPs, one triallelic, in the ELAC2 gene. The objective function is the maximized log likelihood for the model penalized by a multiple of the model's degrees of freedom. Simulated annealing is used to find good solutions. The results show clear non spatial and non pairwise dependences. The great potential of this approach is that categorical phenotypes can be included in the same analysis and association with polymorphisms assessed jointly with the inter locus associations. This is illustrated in the above example with phenotypic data on sex and incidence of prostate cancer.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 160 from 2003 ASHG Annual Meeting

Joint localization of two linked disease genes: Derivation, evaluation, and application of a new method. *J. Biernacka*[1,2]*, L. Sun*[1]*, J. Stafford*[1]*, S.B. Bull*[1,2]. 1) Department of Public Health Sciences, University of Toronto, Toronto, Canada; 2) Samuel Lunenfeld Research Institute, Toronto, Canada.

Methods that simultaneously consider multiple susceptibility genes may increase the power to detect genes involved in the predisposition to complex disorders. This belief has led to increased interest in procedures that test for the existence and/or interaction of secondary genes taking into account the presence of primary genes. Extending the work of Liang et al. (Human Heredity 51: 64-78, 2001), we have developed a model for the simultaneous localization of two susceptibility genes in one region. We derived an expression for expected allele sharing in affected sib pairs at each point across a chromosomal segment containing two susceptibility genes. With this expression for the mean allele sharing, generalized estimating equations (GEE) can be used to estimate the locations of both disease genes simultaneously. We developed an algorithm that uses information on marker IBD sharing in affected sib pairs to estimate the expected IBD sharing in affected sib pairs at two linked disease loci and the locations of the two genes. Furthermore, confidence regions for gene locations can be constructed based on large sample approximations using parameter estimates and a robust estimate of their covariance matrix. Via simulation studies we found that good estimates of disease gene locations can be obtained by this method. Properties of the estimates and confidence intervals, including bias, precision, and confidence interval coverage, have been studied for a range of genetic models. The ability of this method to localize disease genes improves with increased expected allele sharing at the disease genes, increased distance between the disease genes, and increased sample size. We applied the described methods to data from a genome scan for type 1 diabetes (n=263) (Nature Genetics 19: 297-300, 1998) and obtained estimates of two disease gene locations on chromosome 16, estimated to be approximately 50 cM apart. Our results suggest that the proposed method can improve disease gene localization and aid in separating large peaks when two disease genes are present in one chromosomal region.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Identification of a disease susceptibility gene involved only in a subsample of patients. *F. Clerget-Darpoux, P. Margaritte-Jeannin, H. Selinger-Leneman.* INSERM U535, Hosp Paul Brousse, Villejuif, Cedex, France.

A major challenge for Genetic Epidemiology is the identification of genetic factors involved in multifactorial diseases. For these diseases, the number of factors involved and the level of heterogeneity for each factor are unknown.The information available through the marker segregation in affected sib pairs may be sufficient for detecting linkage and thus for indicating a region in which a susceptibility gene lies. However, it does not generally allow for simultaneous inference of linkage and of linkage heterogeneity. We show that the statistics involving heterogeneity parameter are overparameterized and consequently do not provide more power than less parameterized statistics. When a region has been detected by linkage in an affected sib pair sample, the next step aims at searching for association with intragenic polymorphisms within a candidate region in order to identify the genetic risk factor. The same sib pair sample used in the linkage analysis may also be used for the association studies, considering only one of the two sibs (index case). Many recently published studies restricted this association step to the index of sib pairs sharing two parental alleles identical by descent (IBD=2). The underlying argument is the gain of power in presence of genetic heterogeneity. This belief is false. We show analytically that, whatever the degree of heterogeneity and whatever the model underlying the effect of the risk factor in the disease, restricting the association study to the sub-sample of sib pair IBD=2 always causes a loss of power.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 162 from 2003 ASHG Annual Meeting

Evolution of Linkage Disequilibrium for SNP Markers: Estimating Past Demography and SNP Ages. *M. Kimmel*[1], *J. Polanska*[2]. 1) Dept Statistics, Rice Univ, Houston, TX; 2) Inst Autaomation, Silesian Technical Univ, Gliwice, Poland.

Linkage Disequilibrium (LD) has been an intensely studied subject in recent several years. Introduction of Single Nucleotide Polymorphisms (SNPs) and sequencing of the human genome seem to be the turning points for these studies. Ubiquity of SNPs on the hand and the possibility of anchoring them in the human genome sequence on the other contribute in this trend. Why are we interested in LD? Pragmatic reasons include identification of regions with markers appropriate for disease-association studies. However, there also exist basic questions regarding evolution of the modern human genome which may be elucidated by studying LD. One of these questions is the influence of past demographic trends in current LD. Another question is the age of SNPs. Still another is the relative importance of the basic genetic forces for the evolution of LD. Genetic forces responsible for the observed pattern of LD are recombination, genetic drift, mutation and selection. Even if the selective sweeps are not taken into account, joint consideration of the remaining three forces is quite complicated and is usually considered to require simulation methods. These usually are based on the coalescence process and are powerful but computationally intensive. We propose to use a simple model of evolution at a pair of SNP loci, under mutation, genetic drift and recombination. Our model is extremely fast computationally, which makes possible to review a large number of parameter values in a short time. Mutation is modeled using a two-state Markov model. We are able to consider evolution of SNPs under different demographic scenarios. In particular, we explore the bottleneck scenario of demography of early modern humans. We use the same set of SNP data, which was employed by Reich et al. (2001). However in contrast to Reich et al. (2001) we fit the model not only to LD data, but also to genetic drift data. This allows us to reconcile the influences of these two processes. Also, in addition to the demographic parameters, we estimate the ages of all SNPs. As it is seen, our results result in somewhat different conclusions compared to those of Reich et al. (2001).

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 163 from 2003 ASHG Annual Meeting

Identifying SNPs Responsible for a Linkage Signal. *M. Li, G.R. Abecasis, M. Boehnke.* Dept Biostatistics, Univ Michigan, Ann Arbor, MI.

Once genetic linkage has been identified for a complex disease, the next step is often association analysis, in which single-nucleotide polymorphisms (SNPs) within the linkage region are genotyped and tested for association with the disease. If a SNP shows evidence for association, a key question is to what degree the linkage result can be explained by the associated SNP. To answer this question, we are developing a novel statistical method that quantifies the degree of linkage disequilibrium (LD) between the associated SNP and the putative disease locus. We describe a simple parametric likelihood of the marker data conditional on the trait data based on disease penetrances and disease-SNP haplotype frequencies. Special cases of the likelihood include complete LD and linkage equilibrium (LE). We estimate model parameters by maximum likelihood using a simplex method. We propose two likelihood ratio tests to distinguish the relationship of the associated SNP and the disease locus. The first test assesses whether the associated SNP and the disease locus are in LE so that the SNP plays no causal role in the linkage signal. The second test assesses whether the associated SNP and the disease locus are in perfect LD so that the SNP or a marker in complete LD with it can fully account for the linkage signal. LD measures such as D' and r^2 can be estimated from the disease-SNP haplotype frequency estimates. These estimates are of particular interest given incomplete disease-SNP LD. To investigate the performance of our method, we simulate 500 affected sibling pairs under a variety of models with sibling recurrence risk ratio ~ 1.4. Simulation results demonstrate that our method yields accurate estimates of model parameters and LD measures when LD is strong. Accuracy of the estimates can be improved by including unaffected sibling pairs when LD is moderate or weak. For dominant model, our tests have $>$ 85% power to reject LE when r^2 0.25, and $>$ 80% power to reject complete LD when r^2 0.50 at significance level 0.05. Powers are similar for additive model, but lower for recessive model. Our method will be valuable for prioritizing SNPs in searching for disease-susceptibility alleles.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Random Forests, SNP Importance and Complex Traits. *P. Van Eerdewegh, A. Bureau, K. Lunetta, B. Hayward, K. Falls.* Dept Human Genetics, Genome Therapeutics Corp, Waltham, MA.

Complex diseases such as asthma, schizophrenia, diabetes and hypertension are believed to result from interactions between multiple genetic and environmental factors. Association studies of common complex diseases will soon generate genotypic data at thousands of single nucleotide polymorphisms (SNPs) with the goals of identifying genetic factors that increase disease risk and of elucidating the genetic architecture of those traits. Random Forests (Breiman, 2001) has shown promise for identifying polymorphisms associated with complex diseases. A Random Forest is a collection of classification trees grown on bootstrap samples of observations, selecting the best split at each tree node among a random subset of predictors. We have extended the concept of importance of a predictor to capture the joint effects of SNPs and explore the properties of importance measures for multi-locus models of inheritance. In addition, we explored the ability of the Random Forests proximity measures to identify homogeneous subsets of cases in order to resolve heterogeneity among sets of genetic predisposing factors. We used simulation to assess the performance of Random Forests in 100 replicates of, on average, 133 cases and controls genotyped at 67 SNPs on a chromosome segment from a coalescent. A disease model of genetic heterogeneity between two sets of susceptibility factors, each with a pair of epistatically interacting SNPs, was used to simulate the trait. The SNP pairs most often identified were the epistatic pairs; in contrast a Fisher exact test more frequently identified pairs with one SNP from each heterogeneous system. In a replicate where all disease SNPs were identified, clusters created by the proximity measures of Random Forests separated cases carrying risk alleles in different systems of epistatic SNPs. In summary, our simulation studies show that variable importance indices can identify susceptibility SNPs, and suggest that clustering of individuals can identify epistatic interactions between SNPs. The ability of Random Forests to analyze jointly large numbers of SNPs makes it an ideal tool to understand the genetic etiology of complex diseases.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Handedness and schizophrenia susceptibility are paternally linked to a locus on chromosome 2p12-q11. *C. Francks[1], S.H. Shaw[2], S.E. Fisher[1], A.J. Richardson[3], J.F. Stein[3], A.P. Monaco[1].* 1) Wellcome Trust Center for Human Genetics, University of Oxford, Oxford, UK; 2) Illumina Inc., San Diego, CA, USA; 3) Department of Physiology, University of Oxford, Oxford, UK.

Schizophrenia and non-righthandedness are moderately associated, and both traits are often accompanied by abnormalities of asymmetrical brain morphology or function. We have previously found linkage of a locus on chromosome 2p12-q11 to a quantitative measure of handedness. Linkage has also been found of schizophrenia/schizoaffective disorder to this same chromosomal region in separate studies, and this was the most strongly linked region in a recent meta-analysis of 20 genome scans. Now, we have found that in one of our samples (191 reading-disabled sibling pairs), the relative hand skill of siblings was correlated more strongly with paternal than maternal relative hand skill. This led us to re-analyze the 2p12-q11 locus under parent-of-origin linkage models (extended Haseman-Elston regression, variance components, and affected-sib-pair analyses). We found linkage of relative hand skill in the RD siblings to 2p12-q11 with P=0.0000021 for paternal sharing with multipoint regression analysis, and empirical P<0.00001 for paternal sharing with multipoint variance components analysis, whereas the maternally inherited locus was not linked to the trait (P>0.2). Furthermore, in multipoint affected-sib-pair analysis of a schizophrenia dataset that was known to show linkage to 2p12 (241 pairs), we found linkage for paternal sharing with LOD=4.72, P=0.0000016, within 3 centimorgans of the linkage to relative hand skill. Again, maternal linkage across the region was weak or non-significant. The concordance of paternal linkages of schizophrenia and relative hand skill to 2p12-q11 provides evidence that the underlying genetic effects at this locus are directly related. The linkages may be due to a maternally imprinted influence on lateralised brain development that contains common functional polymorphisms.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 166 from 2003 ASHG Annual Meeting

Sex and age dependent effects of chromosomal regions linked to body mass index across 28 years of the Framingham Heart Study. *L.D. Atwood[1,2], N.L. Heard-Costa[1], L.A. Cupples[2], C.S. Fox[3], C.E. Jaquish[4], P.W.F. Wilson[5], R.B. D'Agostino[6]*. 1) Neurology, Boston Univ Sch Medicine, Boston; 2) Biostatistics, Boston Univ Sch Public Health, Boston; 3) Framingham Heart Study, NHLBI, Framingham, MA; 4) NHLBI, Bethesda, MD; 5) Medicine, Boston Univ Sch Medicine, Boston; 6) Mathematics, Boston Univ, Boston.

We have reported significant linkage of body mass index (BMI) to chromosomes 6 and 11 across six measurements, covering 28 years of the Framingham Heart Study. These results were on all individuals available at each exam, thus the sample size varied from exam to exam. To explore the effect of sample size variation we constructed six subsets; for each exam, individuals were only included if they were measured *at all six exams*. This strategy preferentially removed older individuals who died before reaching the sixth exam, thus the new datasets were smaller and younger than the full datasets. We performed variance components linkage analysis on the new datasets (with the same individuals for each exam, n=1114) and on their sex-specific subsets. BMI linkages to chromosomes 6 and 11 remained significantly linked in these analyses, consistent with earlier results. Surprisingly, the maximum lodscore for exam 1 on chromosome 10 increased from 0.97 in the older full dataset to **4.23** in the smaller subset with younger individuals. A randomization test of this difference in maximum lodscores was highly significant (p=0.0007), implying stronger linkage in younger individuals. Sex-specific linkage of BMI showed that men were more strongly linked to chromosomes 1, 16 and 18 than women, and women were more strongly linked to chromosomes 7 and 10 than men. In each region, randomization tests in at least three of the six exams revealed significant ($p< 0.05$) differences between the sex-specific maximum lodscores. The strongest sex-specific linkage was of men to chromosome 16 with maximum lodscores 2.70, 3.00, 3.42, 3.61, 2.56 and 1.93 for exams 1-6 respectively.

We conclude that there are sex- and age-dependent linkages for obesity to multiple chromosomal regions and that multiple measures taken during adulthood help to characterize these effects.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 167 from 2003 ASHG Annual Meeting

A Major Susceptibility Locus for Gallbladder Disease is on Chromosome 11p in Mexican Americans. *R. Duggirala*[1], *G.D. Dodd*[2], *S. Fowler*[3], *J. Schneider*[1], *R. Arya*[3], *A.K. Diehl*[3], *L. Almasy*[1], *P. O'Connell*[4], *M.P. Stern*[3], *J. Blangero*[1]. 1) Genetics, SFBR, San Antonio, TX; 2) Radiology, UTHSCSA, San Antonio, TX; 3) Medicine, UTHSCSA, San Antonio, TX; 4) Human Genetics, VCU, Richmond, VA.

Gallbladder disease (GBD) is an economically burdensome digestive disease, which often clusters with diseases such as obesity and type 2 diabetes. The major susceptibility loci for GBD in humans are yet to be identified. In this study, we performed a multipoint variance components linkage analysis to identify susceptibility loci for GBD in Mexican Americans. Ultrasound-based GBD data and other phenotypic information were collected from 741 individuals (39 families) as part of the San Antonio Family Gallbladder Disease Study (SAFGS), a sub-study of the San Antonio Family Diabetes Study (SAFDS). Of the 741 participants, 349 individuals (mean age = 48 years, 61% females, and 34% diabetics) were in the genotyped data set (~10 cM Map) of the SAFDS. Two GBD phenotypes were defined for the analyses: (1) GBD1, the cases of cholecystectomies due to stones only confirmed by ultrasound and (2) GBD2, the GBD1 cases plus the newly diagnosed stone carriers by ultrasound. The prevalence of GBD1 and GBD2 are 18% and 32%, respectively. After adjusting for age and sex effects, heritability of GBD1 was high ($h^2 = 0.50$), and it was low for GBD2 ($h^2 = 0.19$). Our preliminary linkage analyses suggest that the GBD1 (i.e., symptomatic gallstone disease) is more informative for linkage than GBD2, and only chromosomal regions with multipoint LOD scores 1.9 for GBD1 were discussed below. The strong evidence for linkage of GBD1 (LOD = 4.1) occurs at marker D11S1984 near p-ter on chromosome 11p. Suggestive evidence for linkage occurs near markers D6S1035 (LOD = 2.1) and D10S245 (LOD = 2.6). After correcting for additional covariate effects of diabetes and waist circumference, evidence for linkage near marker D11S1984 continues to be significant (LOD = 3.5). In these analyses, in addition to markers D6S1035 (LOD = 2.3) and D10S245 (LOD = 3.0), marker D8S270 region exhibits suggestive evidence for linkage (LOD = 2.4). In sum, we found a major locus for GBD on chromosome 11p near marker D11S1984 in Mexican Americans.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 168 from 2003 ASHG Annual Meeting

A common mutation in the *RET* gene in all forms of Hirschsprung disease (HSCR). *A.S. McCallion[1], M.E. Portnoy[2], E. Grice[1], C. Cashuk[1], E. Emison[1], E.D. Green[2], A. Chakravarti[1].* 1) McKusick-Nathans Inst Gen Med, McKusick - Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 2) NHGRI, NIH, Bethesda.

Hirschsprung disease (HSCR), or congenital aganglionosis with megacolon, is a model multigenic disorder. A variety of clinical, genetic and animal model studies have identified more than a dozen genes which, when mutant, participate as one of many factors in human disease expression. However, there is increasing evidence that mutations in the RET gene are central to the genesis of most forms of HSCR and that its effect is epistatic to EDNRB mutations. As many as 90% of HSCR families demonstrate linkage to the RET locus. However, coding sequence mutations are identified with a much lower frequency (50%), suggesting the existence of non-coding, potentially regulatory mutations of RET. To determine the nature of disease-causing mutations in non-coding sequence at RET we have conducted comparative sequence analysis of 300kb in 11 vertebrate species, identifying 20 highly conserved sequences. We have also identified 2 novel genes, within 100 kb, whose temporal/spatial expression overlaps that of RET. We examined the effect of 19 non-coding SNPs in and around the conserved non-coding regions of RET in 5 overlapping patient populations: simplex and multiplex HSCR families; those with and without identified mutations; and syndromic [Down syndrome] families with a co-incident presentation of HSCR. We show that 5 non-coding SNPs, located 5 kb 3 to exon 1 extending to and including exon 2, are over-transmitted at a rate of nearly 81% (range 77-85%) for all five patient populations (TDT p values, $P 3 \times 10^{-9}$ to $P 3 \times 10^{-11}$). These data establish the centrality of a relatively common haplotype within RET in the genesis of nearly all forms of HSCR. Functional analyses of wild-type and deletion mutations in model organisms are being generated to test our observations (See abstract Grice et al.).

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 169 from 2003 ASHG Annual Meeting

Mutation in the RET genomic sequence between the transcription start site and exon 2 as a major contributor to the development of Hirschsprung disease. *R.M.W. Hofstra[1], G. Burzynski[1], I. Nolte[2], J. Osinga[1], I. Ceccherini[3], B. Twigt[1], A. Brooks[4,1], J. Verheij[5], I. Plaza-Menacho[1], C.H.C.M. Buys[1].* 1) Medical Genetics, Univ Groningen, The Netherlands; 2) Medical Biology, Univ Groningen,Groningen, The Netherlands; 3) Laboratorio di Genetica Molecolare, Istituto G. Gaslini, 16148 Genova, Italy; 4) Clinical Genetics, Univ. Rotterdam, The Netherlands; 5) Clinical Genetics, Univ. Groningen, The Netherlands.

Hirschsprung disease (HSCR) is a congenital disorder characterized by intestinal obstruction caused by the absence of the enteric ganglia along a variable length of the intestinal tract. HSCR is thought to be a multifactorial disease with low sex dependent penetrance with the RET proto-oncogene as the major susceptibility gene. Other genes known to be involved in HSCR participate either in the RET or the endothelin signaling pathways. RET mutations can be identified in 50% of familial cases, but only 10%-20% of sporadic cases, that constitute up to 80% of all the HSCR patients. We typed 11 markers, four microsatellites and seven SNPs, in and around the RET locus in DNA from 149 Dutch, mostly sporadic, HSCR patients for whom no mutation in the known genes had been found and their parents. Strong association was found for the five markers that lie in the 5 region of the gene (SNP-5 p=1.210-21; SNP-1 p=5.910-7; SNPint1A p=4.810-16; SNPint1B p=0.015; SNPex2 p=4.210-21). TDT tests also show that the largest differences in transmission of alleles are observed at the same five marker loci (SNP-5 p=1.610-14; SNP-1 p=6.710-8; SNPint1A p=5.710-11; SNPint1B p=0.040; SNPex2 p=3.610-15). Haplotype reconstruction of these five markers using an EM algorithm shows that 59.6% of our patients share a common haplotype compared to 20.7% of the controls (p=2.110-13). These findings provide evidence that RET does play a crucial role in the disease even when no RET mutation is found. Furthermore, we show that a single ancestral RET haplotype exists in our sporadic HSCR patients, suggesting that the Dutch HSCR population is derived from a common ancestor. Our data furthermore make it likely that the unknown functional disease variant lies between transcription start site and exon 2.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Exact and approximate multipoint linkage analysis with GENEHUNTER 3.0. *K. Markianos*[1], *A. Katz*[1], *L. Kruglyak*[1,2]. 1) Dept Human Biol, Box 19024, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Howard Hughes Medical Institute.

We have implemented a new computational approach for rapid multipoint linkage analysis in the software package GENEHUNTER 3.0. For large pedigrees, the current implementation is thousands of times faster than the previous release, and allows analysis of substantially larger families. We do not restrict the pedigree structures analyzed or the number of missing ancestors in the pedigree. However, we achieve high gains in efficiency for pedigrees where most peripheral nuclear families are fully genotyped (here we call peripheral families the nuclear families whose children are the last generation in the pedigree).

For exact inheritance reconstruction we classify the single point and multipoint probability distributions into independent sets using observed marker information. In addition to the exact calculation, we provide an option for approximate reconstruction. The approximations are appropriate for use with dense marker maps.

We present detailed comparisons between GENEHUNTER 3.0 and other analysis programs, as well as examples that illustrate the impact of missing ancestors and pedigree structure on the efficiency of the approach.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 171 from 2003 ASHG Annual Meeting

A Combined Linkage-Physical Map of The Human Genome. *X. Kong[1], K. Murphy[2], T. Raj[2], C. He[1], P.S. White[2], T.C. Matise[1]*. 1) Rutgers U., Piscataway, NJ; 2) Children's Hospital of Philadelphia, Philadelphia, PA.

Completion of sequencing and assembly of the human genome has allowed us to construct a combined linkage-physical map that integrates polymorphic marker genotyping data with DNA sequence data. The meiotic data consists of 16000 markers genotyped in the CEPH pedigrees plus 5136 markers genotyped in deCODE's Icelandic families. We believe this set represents the largest combined collection of genotype data. The physical locations of the PCR-based markers were determined by searching for sequence homology with a modified version of e-PCR, using the current genome assembly (NCBI Build 33). A unique physical position could be determined for 70% of the markers in our set.

To build the combined linkage-physical map, we began with a version of the deCODE linkage map modified to match the current genome assembly. In the initial mapping stages, only markers with known physical positions were used and were added to the map only if the results of linkage analysis agreed with physical position. Markers with no physical position were added in later steps using only linkage information. Finally, markers whose linkage positions did not match physical position were re-evaluated against the more complete map. Those which still had discordant positions were not incorporated into the map. The resulting map provides both linkage map coordinates (in cM) as well as physical positions. Multiple versions of the map are available, using decreasing lod thresholds to allow incorporation of more markers into the map. Although here the statistical support is reduced, the concordancy between linkage and physical data provides added support for marker order.

This set of combined genotype data has been incorporated into the MAP-O-MAT web-based mapping server (http://compgen.rutgers.edu/mapomat) and can be utilized for custom mapping queries. A new function has also been added to MAP-O-MAT, allowing users to identify the map position on the deCODE map of any marker in this combined set. Our maps will also be integrated into the eGenome genomics resource (http://genome.chop.edu).

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 172 from 2003 ASHG Annual Meeting

An associated interval approach for identifying candidate causal variants underlying altered activity of the ABCB1 multi-drug resistance gene. *D. Goldstein[1], N. Soranzo[1], G. Cavalleri[1], N. Wood[2], S. Sisodiya[2].* 1) Biol/Galton Lab/Wolfson House, Univ Col London, London, United Kingdom; 2) Institute of Neurology, Univer Col London, London, United Kingdom.

The difficulty of fine localising the polymorphisms responsible for genotype-phenotype correlations is as an important constraint in genetic association studies. One example is the C3435T polymorphism in the ABCB1 gene, encoding P-glycoprotein, a efflux pump implicated in multi-drug resistance. This polymorphism has been clearly associated with P-glycoprotein activity, but sits within an extended block of linkage disequilibrium (LD) suggesting that this silent polymorphism may not itself be causal. Here we identify a set of candidate causal variants in the ABCB1 gene by first using patterns of LD throughout the gene to define an associated interval surrounding the C3435T polymorphism, which delimits the region within which the causal variant is likely to reside. We then re-sequenced derived and ancestral chromosomes at the C3435T polymorphism through the 25 kilobase associated interval. Using this strategy we found 24, mostly new single nucleotide polymorphisms (SNPs) and one 5-bp indel polymorphism that differentiate the ancestral and derived C3435T chromosomes. We show that three of these SNPs are strongly associated with C3435T, suggesting that the variant(s) responsible for influencing P-glycoprotein may be included within this set of four polymorphisms. Of these three additional variants, one shows a significant association with multidrug resistance in epileptic patients (P=0.037), representing therefore a second candidate (in addition to C3435T) variant underlying altered P-glycoprotein activity. We further assess evidence for the possible function of these polymorphism through the extent of sequence conservation in multiple mammalian species.We conclude that the strategy presented here will be an effective general strategy for identifying a candidate set of causal variants once a polymorphism has been associated with a phenotype of interest, especially in cases such as the C3435T polymorphism which show high penetrance for intermediate phenotypes.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 173 from 2003 ASHG Annual Meeting

Coalescent-based LD fine-mapping narrows location interval and identifies mutation heterogeneity. *D.J. Balding[1], A.P. Morris[2], J.C. Whittaker[1], L.K. Hosking[3], C.F. Xu[3].* 1) Dept Epidemiology & Public Health, Imperial College London, United Kingdom; 2) Welcome Trust Centre for Human Genetics, Oxford, United Kingdom; 3) Discovery Genetics, GlaxoSmithKline R & D, Stevenage, United Kingdom.

Hosking et al (Pharmacogenomics J, 2:165-175, 2002) examined genotypes at 32 SNP loci in an 880 Kb region flanking CYP2D6. They identified a 390 Kb interval displaying strong LD with predicted poor-metaboliser phenotype. We applied COLDMAP, a Bayesian, coalescent-based LD fine-mapping algorithm to the same data, yielding a 95% location interval of only 188 Kb and identifying CYP2D6 as the most likely transcript. Strikingly, our interval excludes 5 SNPs which individually display genome-wide significant association, including the SNP showing the strongest LD. COLDMAP also correctly distinguishes all 72 case chromosomes bearing the CYP2D6 major mutation from the 10 bearing minor mutations. Similarly, the algorithm is succesful at distinguishing controls misclassified as cases. Repeating the analyses with various SNP subsets reveals that, while effective SNP selection can preserve much of the genetic variation, there is some loss of precision for fine-mapping, even with the most effective SNP-selection strategy.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 174 from 2003 ASHG Annual Meeting

A new genome screen for autism based on the posterior probability of linkage (PPL) and incorporating language-based phenotypes finds evidence of linkage to several genomic locations, each supported by independent sources of information. *V.J. Vieland[1], V. Sheffield[1], T. Wassink[1], J. Beck[1], R. Goedken[1], D. Childress[2], J. Piven[2].* 1) Univ Iowa, Iowa City, IA; 2) Univ North Carolina, Chapel Hill NC.

We have recently completed a new genome screen for autism. Included in this study were 59 nuclear families with at least 2 autistic children each, which had been previously analyzed [CLSA, 1999]; as well as 60 similar newly collected families. In recognition of a growing body of evidence that language-development information may be relevant to autism genetics, and increasing evidence that autism and specific language impairment (SLI) may share some common etiology, we have included phenotypic data on language development in the parents, and also classified families based on an extreme language-delay phenotype in the affected children. Finally, we have analyzed the genome using the PPL (posterior probability of linkage), which was specifically designed for analyses of this type. The PPL is on the probability (0,1) scale, with 1 indicating certain linkage and 0 indicating no linkage; the prior probability of linkage is set to 0.02, with PPLs > 0.02 indicating (some degree of) evidence in favor of linkage. Trait parameters are treated as nuisance parameters and integrated out so that the method is essentially model-free in the usual sense. Out of 564 microsatellite markers, all but 10 or so gave either evidence against linkage or extremely weak evidence in favor of linkage. The markers showing non-negligible evidence in favor of linkage represent 6 distinct genomic regions, with peak PPLs: 70% for CENTG24757 (chrom 2@249.22 cM); 70% for 16S3396 (16@63.78); 31% for GATA149E11 (20@12.12); 21% for KLF12TTTA (13@55.60); 12% for D7S821 (7@109.120); and 10% for MECP2 (X@102.35). While only time will tell which of these is real, it is of great interest to note that every one of these locations is supported by independent evidence, based on individuals with chromosomal abnormalities, on the location of compelling candidate genes, on other linkage studies of autism, or on linkage studies of SLI.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 175 from 2003 ASHG Annual Meeting

Unmasking Kabuki syndrome: chromosome 8p22-8p23.1 duplication revealed by comparative genomic hybridization and BAC-FISH. *J.M. Milunsky*[1, 2, 3], *X.L. Huang*[1]. 1) Center for Human Genetics, Boston University School of Medicine, Boston, MA; 2) Department of Pediatrics, Boston University School of Medicine, Boston, MA; 3) Department of Genetics and Genomics, Boston University School of Medicine, Boston, MA.

Kabuki syndrome (KS) is a multiple congenital anomalies/mental retardation syndrome characterized by craniofacial, dermatoglyphic, and skeletal abnormalities. More than 350 cases have been reported worldwide. The etiology of KS is undetermined. Although several cases with KS features have been reported with different chromosome anomalies, none have had an autosomal cytogenetic aberration in-common. The range of multiple anomalies and mental retardation in KS raise the possibility of a contiguous gene syndrome. We employed comparative genomic hybridization (CGH) in 6 unrelated patients who met the cardinal diagnostic criteria for KS. CGH revealed an 8p22-8p23.1 duplication that was confirmed using BAC-FISH in all cases. The duplicated region has been delimited to approximately 3.5 Mb. 2/2 mothers of KS patients were found to have a heterozygous submicroscopic inversion at 8p23.1. 6/6 KS patients had the same inversion at 8p23.1 and have a duplication involving the 8p22-8p23.1 region on the same chromosome separated by a normal segment. 1/20 controls had a larger submicroscopic inversion at 8p22-8p23.1. None of the controls had duplication of this region detected by CGH or BAC-FISH. Two previously described 8p- Olfactory receptor gene clusters flank this region and are involved in an inversion polymorphism (Giglio et al. 2001). This inversion likely mediates unequal meiotic crossovers resulting in a tandem dup(8p). We propose that KS may be a contiguous gene duplication syndrome of 8p22-8p23.1 whose molecular mechanism appears to be gene dosage, similar to CMT1A. As the 6 patients with KS are from different racial groups, this duplication is likely to represent a common etiologic basis for this disorder.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Large-scale deletions versus truncating mutations at the *ZFHX1B* glocus in Mowat-Wilson syndrome: genotype-phenotype correlations. *Y. Espinosa-Parrilla, A. Munnich, S. Lyonnet, J. Amiel.* INSERM U-393, Hôpital Necker-Enfants malades, Paris, France.

Mowat-Wilson syndrome (MWS) is a recently recognized multiple congenital anomaly-mental retardation syndrome (MCA-MR) caused by haploinsufficiency of the zinc finger homeobox 1 B gene (*ZFHX1B*) at 2q22. All patients share at least a distinctive facial gestalt and moderate to severe MR. We looked for mutations or deletions at the *ZFHX1B* locus by DHPLC, FISH, semiquantitative-PCR and microsatellite analysis in 39 patients with suggestive facial dysmorphism, MR and normal chromosomes at a 400-band resolution. Sixteen truncating mutations and 8 large-scale deletions were identified in 24 patients (8 previously reported). Ten mutations and 6 deletions could be proven to have occurred *de novo*. Either a paternal or a maternal origin of the deletion could be found in 4 and 2 cases respectively. Regarding the phenotype of patients with an identified *ZFHX1B* abnormality, postnatal microcephaly was found in 83%, Hirschsprung disease (HSCR) in 74%, growth retardation in 71%, agenesis of the corpus callosum in 70%, epilepsy in 63%, heart defects (HD) in 38%, renal anomalies in 25%, and 71% of the males had hypospadias. Although the overall spectrum of malformations was common to mutated and deleted patients, some features were far more frequent in the group of patients with a deletion encompassing the whole *ZFHX1B* locus. Interestingly, whereas HSCR, hypospadias and HD were found in all deleted patients, these features were found in 60%, 63% and as little as 7% of patients with truncating mutations respectively. Among the 15 cases with no detectable mutation/deletion, the diagnosis of MWS remained very likely in 6 cases. Two patients were suspected of Goldberg-Shprintzen syndrome and 7 had the MWS clinical spectrum of malformations but a different facial gestalt. In conclusion we have identified a *ZFHX1B* defect in 24/30 patients with a MWS phenotype (80%) and delineated differences in the frequency of malformations between *ZFHX1B* mutated or deleted patients. Our results strengthen the importance of deletion screening particularly when MWS is associated with HSCR, HD and urogenital anomalies.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 177 from 2003 ASHG Annual Meeting

SALL4 mutations result in a range of clinically overlapping phenotypes, including Okihiro syndrome, Holt-Oram syndrome, Acro-Reno-Ocular syndrome and patients previously reported to represent Thalidomide Embryopathy. *J. Kohlhase[1], L. Schubert[1], M. Liebers[1], R.C.M. Hennekam[2], A. Rauch[3], K. Becker[4], S.N. Mohammed[5], M. Wright[6], M.C. Hannibal[7], R. Newbury-Ecob[8], W. Reardon[9].* 1) Institute of Human Genetics, University of Goettingen, Germany; 2) Departments of Pediatrics and Clinical Genetics, Academic Medical Center, University of Amsterdam, The Netherlands; 3) Institute of Human Genetics, University Erlangen-Nuremberg, Germany; 4) Kennedy-Galton Centre, Northwick Park Hospital, Harrow, UK; 5) Genetics Centre, Guys Hospital, London, UK; 6) Institute of Human Genetics, International Centre for Life, Newcastle-upon-Tyne, UK; 7) Division of Genetics and Development, Department of Pediatrics, University of Washington, Seattle, WA, USA; 8) Department of Clinical Genetics, St Michael's Hospital, Bristol, UK; 9) National Centre for Medical Genetics, Our Lady's Hospital for Sick Children, Crumlin, Dublin, Ireland.

Okihiro syndrome results from mutation in the putative zinc finger transcription factor gene *SALL4* on chromosome 20q13. Since there is considerable overlap of Okihiro syndrome with Holt-Oram syndrome, a condition in part resulting from mutation of the *TBX5* locus, as well as Acro-Renal-Ocular syndrome, we analyzed further patients with the clinical diagnosis of Holt-Oram syndrome, Okihiro syndrome and Acro-Renal-Ocular syndrome for *SALL4* mutations. We identified a novel *SALL4* mutation in one family where the father was originally thought to present with thalidomide embryopathy and had a daughter with a similar phenotype. We also found two novel mutations in two German families originally diagnosed as Holt-Oram syndrome and two further mutations in two out of three families carrying the diagnosis Acro-Renal-Ocular syndrome as well as two further mutations in patients with Okihiro syndrome. Our results show that some cases of thalidomide embryopathy might be due to *SALL4* mutations, resulting in an increased risk for likewise affected offspring. Furthermore we confirm the overlap of Acro-Renal-Ocular syndrome with Okihiro syndrome also on the molecular level and expand the phenotype of *SALL4* mutations.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 178 from 2003 ASHG Annual Meeting

A new genomic disorder mediated by low-copy repeats? Fifty microdeletions identified in 112 patients with Sotos syndrome. *N. Kurotaki[1], N. Harada[4,5,6], J.F. Cheng[7], J.R. Lupski[1,2,3], N. Matsumoto[4,5]*. 1) Dept. of Molecular and Human Genetics, Baylor College of Medicine,Houston, TX; 2) Dept. of Pediatrics, Baylor College of Medicine, Houston, TX; 3) Texas Children Hospital, Houston, TX; 4) Dept. of Human Genetics, Nagasaki University School of Medicine, Nagasaki, Japan; 5) CREST, Japan Science and Technology Corporation, Kawaguchi, Japan; 6) Kyushu Medical Science Nagasaki Laboratory, Nagasaki, Japan; 7) Genome Sciences Dept, Lawrence Berkeley National Laboratory, CA.

We previously showed that haploinsufficiency of the NSD1 gene is the major cause of Sotos syndrome (SoS), and submicroscopic deletions within 5q35 involving NSD1 were found in about a half (20/42) of our patients examined. Since the first report, additional 70 SoS cases consisting of 53 Japanese and 17 non-Japanese patients have been studied. Out of the 112 cases analyzed, 50 microdeletions (44.6 %) and 16 point mutations (14.3 %) were found. A large difference in the frequency of microdeletions between Japanese and non-Japanese patients was noted: 49 (51.6 %) out of the 95 Japanese patients and only one (5.9 %) of the 17 non-Japanese carried microdeletion. A physical map has been constructed to better characterize these micordeletions. Most were confirmed to be identical in size by FISH analysis. As a result, 46 Japanese patients were deleted for an identical ~0.7Mb genomic region. We identified an ~95% identical, 140 kb LCR, SoS-REP, in regions flanking proximal and distal SoS common deletion breakpoints. SoS-REP are with one direct and three inverted orientations between proximal and distal copy. Our data suggest that Sotos syndrome is a new genomic disorder. Moreover, the different microdeletion frequencies between Japanese and non-Japanese cases may potentially be caused by either patient-selection bias or population specific polymorphic LCR copy number.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

MUTATION ANALYSIS IN SOTOS SYNDROME. *F. Faravelli[1], M. Cecconi[1], F. Forzano[1], M. Malacarne[1], S. Cavani[1], C. Baldo[1], D. Milani[2], A. Selicorni[2], M. Silengo[3], G.B. Ferrero[3], G. Scarano[4], M. DellaMonica[4], L. Memo[5], C. Pantaleoni[6], R. Pallotta[7], A. Renieri[8], D. Concolino[9], F. Dagna Bricarelli[1], M. Pierluigi[1], M. Grasso[1]*. 1) Lab Human Genetics, Ospedale Galliera, Genova, Italy; 2) Clinica Pediatrica DeMarchi, Milano, Italy; 3) Universit di Torino, Torino, Italy; 4) Azienda Ospedaliera Gaetano Rummo, Benevento, Italy; 5) Ospedale dei Battuti, Treviso, Italy; 6) Istituto Besta, Milano, Italy; 7) Universit G.D'Annunzio, Chieti, Italy; 8) Nuovo Policlinico LeScotte, Siena, Italy; 9) Universit Magna Grecia , Catanzaro, Italy.

Sotos syndrome is characterized by the association of childhood overgrowth (height and head circumference above the 97th centile), advanced bone age and typical facial features. Developmental delay is frequently associated. Some overlap exists with other overgrowth conditions, in particular with Weaver syndrome.The gene responsible for Sotos syndrome was recently identified (Kurotaki et al., 2002) and mutation testing revealed some ethnic difference in the prevalence of different types of mutations. In particular, microdeletions involving the gene NSD1 appear to be very frequent in Japanese patients with Sotos syndrome, but this data was not confirmed in European series (Douglas et al., 2003, L. Clech et al., 2003). Douglas et al.(2003) suggested a phenotypic classification for overgrowth patients and reported mutations in NSD1 gene in patients with Weaver syndrome too, suggesting the presence of genotype-phenotype correlation. We report data on mutation analysis of the NSD1 gene in 67 patients, 30 of which fully analyzed, 37 under analysis: 45 were sporadic cases of Sotos syndrome, 7 were Sotos-like, 12 were affected by overgrowth, 3 cases had a clinical suspicion of Weaver syndrome. FISH analysis revealed only one 5q35 deletion out of the 50 patients examined, thus demonstrating that microdeletion of 5q35 is not a frequent cause of SS in Italian patients. Molecular analysis was performed by DHPLC and direct sequencing. 8 novel stop mutations and the polymorphisms detected will be described and genotype-phenotype correlation of our series di.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 180 from 2003 ASHG Annual Meeting

Variability in clinical phenotype despite common chromosomal deletion in Smith-Magenis syndrome [del(17)(p11.2p11.2)]. *J.R. Lupski*[1, 2, 3, 4], *C.J. Shaw*[1, 3], *P. Stankiewicz*[1, 3], *L. Potocki*[1, 3, 4]. 1) Department of Molecular & Human Genetics; 2) Department of Pediatrics; 3) Baylor College Medicine, Houston, TX; 4) Texas Children's Hospital, Houston.

Smith-Magenis syndrome (SMS) is a multiple congenital anomalies and mental retardation syndrome associated with an interstitial deletion within 17p11.2. In the majority of patients, the deletion is derived from non-allelic homologous recombination between low-copy repeat gene clusters during either maternal or paternal gametogenesis. This report delineates the phenotypic features in a cohort of 58 persons with SMS, and compares features of patients with the common microdeletion to those of patients with variable sized deletions, and the three previously reported patients who harbor a mutation in *RAI1* (retinoic acid induced 1). Fifty eight persons with SMS were enrolled in a 5-day clinical protocol at the Texas Children's Hospital. Each patient had a cytogentically evident deletion in 17p11.2. Of the 51 patients in whom the molecular extent of the deletion could be delineated by pulsed-field gel electrophoresis and/or FISH, 39 (76%) had the common SMS deletion. Smaller or larger deletions were seen in 12% and 10% of patients, respectively, and 1 patient had a complex chromosomal rearrangement including a deletion in 17p11.2. All patients had impaired cognitive and adaptive functioning, and had at least one objective measure of sleep disturbance. Common features seen in greater than 50% of patients include short stature, ophthalmologic and otolaryngologic anomalies, hearing impairment, abnormal EEG, and scoliosis. Cardiac and renal anomalies were seen in 45% and 19% of patients, respectively. There are no statistically significant differences in the incidence of these abnormalities in patients with the common deletion compared to those patients with smaller or larger sized deletions. Despite a common deletion size in 76% of patients, the only constant objectively defined features are sleep disturbances, low adaptive functioning, and mental retardation. There is no pathognomonic clinical feature, no characteristic cardiovascular defect, renal anomaly, otolaryngologic or ophthalmic abnormality in SMS.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

The emerging clinical phenotype of the dup(17)(p11.2p11.2) syndrome: the homologous recombination reciprocal of the Smith-Magenis microdeletion. *L. Potocki*[1, 3, 4], *C.J. Shaw*[1, 3], *P. Stankiewicz*[1, 3], *J.R. Lupski*[1, 2, 3, 4]. 1) Department of Molecular and Human Genetics; 2) Department of Pediatrics; 3) Baylor College of Medicine, Houston, TX; 4) Texas Children's Hospital, Houston.

Nonallelic homologous recombination within region-specific low-copy repeats is known to give rise to DNA rearrangements associated with many genetic disorders. Smith-Magenis syndrome (SMS) is a well characterized multiple congenital anomalies syndrome due to a heterozygous deletion within 17p11.2. We previously described 7 persons with dup(17)(p11.2p11.2) which is the homologous recombination reciprocal of the SMS deletion, and commented on the relatively mild phenotype as compared to individuals with del(17)(p11.2p11.2). Herein we present the molecular and clinical data on 6 patients with dup(17)(p11.2p11.2) who were evaluated through a multidisciplinary clinical protocol in the General Clinical Research Center at Texas Children's Hospital. Each patient harbors a *de novo* duplication of 17p11.2 of the same size as determined by pulsed-field gel electrophoresis and/or FISH. The parental origin was determined in all patients. Clinical features seen in these patients include dysmorphic craniofacial features, short stature, hypotonia and failure to thrive, submucous palatal cleft and oropharyngeal dysphasia, dental anomalies, hearing impairment, neurocognitive impairment, and behavioral problems including autistic, aggressive and self-injurious behavior. One patient has a structural cardiac anomaly, yet none have renal or CNS malformations. There is marked variability in the physical features and behavioral profile between patients with dup(17)(p11.2p11.2). This variability cannot be attributed to duplication size, and does not seem to be associated with parent-of-origin effect. Only a few patients with dup(17)(p11.2p11.2) have been reported. It is predicted that the incidence of this duplication is equal to that of SMS; however as this duplication is difficult to detect by routine cytogenetic analysis, many of these patients are not ascertained. Systematic clinical evaluation of several more patients will be necessary to determine the features most characteristic of this microduplication syndrome.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

A locus for Dandy-Walker malformation maps to chromosome 3q. *I. Grinberg[1], H.H. Ardinger[2], C. Prasad[3], W.B. Dobyns[1], K.J. Millen[1]*. 1) Dept Human Genetics, Univ Chicago, Chicago, IL; 2) Division of Genetics, Kansas U, Kansas City, KS; 3) Dept Clin Genetics/Metabolism, Childrens Hosp,Winnipeg, MB.

Dandy-Walker malformation (DWM) is a common congenital brain malformation that consists of hypoplasia of posterior greater than anterior cerebellar vermis, cystic dilation of the fourth ventricle and often hydrocephalus. Affected individuals have a variable phenotype that may include cognitive, motor and visual handicaps, ataxia, spasticity and epilepsy. Although several cytogenetic abnormalities have been associated with DWM, no genes have yet been implicated in its pathogenesis and its etiology remains unknown. Here we report five patients with *de novo* interstitial deletions of chromosome 3q2 mapped by FISH, which when analyzed with a previously published 3q2 deletion patient localize a 1.9 Mb minimal critical region for DWM. MRI and/or CT scans of these patients show severe classical DWM in four of six patients (two of whom also have mild brainstem hypoplasia), mild DWM in one of six, and an uncertain result due to a suboptimal CT scan in the remaining patient. One of the six deletion patients also has hydrocephalus and two have blepharophimosos-ptosis-epicanthus inversus (BPES) syndrome, likely the result of deletion of the *FOXL2* gene in 3q23. Fourteen genes are found in the 1.9 Mb DWM critical region. *In situ* hybridization expression analysis in mouse embryos identified three exceptional candidate genes expressed either in the developing cerebellum or throughout the brain. Microdeletion and sequencing analysis of these genes in DWM patients with no known cytogenetic abnormalities should allow the identification of the first DWM causative gene, and provide the first insight into the genetic and developmental basis of this important CNS malformation.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Computer-based classification results of an extensive set of dysmorphic faces. *S. Boehringer[1], G. Gillessen-Kaesbach[1], A. Tewes[2], H.S. Loos[2], R.P. Würtz[2], B. Horsthemke[1], D. Wieczorek[1].* 1) Institut fr Humangenetik, Universittsklinikum Essen, Germany; 2) Institut fr Neuroinformatik, Bochum, Germany.

An extensive set of digital images (n=147) of faces from patients with certain syndromes was collected. These syndromes include Mucopolysaccharidosis type III (n=10), Cornelia de Lange (n=12), Fragile X (n=12), Prader- Willi (n=12), Williams-Beuren (n=13), 5p- (n=16), 22q- (n=23), Noonan (n=18), Sotos (n=18) and Smith-Lemli- Opitz (n=13) syndrome. The analysis is performed in two steps, first obtaining a numeric decomposition of a facial image using Gabor-Wavelet transformations and second applying statistical classification methods. We show results for kth-nearest neighbour (kNN), support vector machines (SVM) and linear discriminant analysis (LDA). Prior to performing SVM and LDA we employed a principle component analysis to reduce the complexity of the data set. This step resulted in 30 components out of 3840 covariates in the original data set. The error rates as measured by cross validation are 37%, 49% and 31% for kNN, SVM and LDA, respectively. These results indicate that the methods presented can be helpful to guide the diagnostic process when comparing them to the 90% error rate of a random choice. Reconstructions of average or typical images of faces for a given syndrome allow to comprehend the decision process of a classification algorithm.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 184 from 2003 ASHG Annual Meeting

Clinical and Molecular Characterization of a Unique Familial Disorder: Proximal Myopathy, Paget disease of Bone and Frontotemporal dementia. *V.E. Kimonis[1], J. Wymer[1], S. Mumm[2], S. Mehta[1], A. Pestronk[3], M. Whyte[2], G. Watts[1].* 1) Genetics, CHB, Harvard Medical School, Boston, MA; 2) Division of Bone and Mineral Diseases, Washington University, St Louis, MO; 3) 3Dept. of Neurology, Washington Univ. School of Med., St Louis, MO.

We have delineated a new limb-girdle myopathic disorder associated with Paget disease of bone (PDB) and frontotemporal dementia (FTD. Of 87 affected individuals in 13 families, 75 (86%) had progressive proximal limb girdle type of muscle weakness resulting in early demise from respiratory failure. EMG and muscle biopsy revealed myopathic changes, with 25% of individuals showing rimmed vacuolar muscle fibers suggestive of 'inclusion body myopathy'. Paget disease of the bone caused by overactive osteoclasts in 45 (52%) individuals was associated with trabecular coarsening,and cortical thickening, with pain primarily of the spine and hip, elevated alkaline phosphatase, and elevated urine pyridinoline/ deoxypyridinoline levels. Frontotemporal dementia associated with relative sparing of memory and impairment of executive skills occurred in 25 (29 %) individuals at a mean age of 54 y. We have previously demonstrated linkage to 9p 13.3-p12 in 4 families (Kovach et al 2001) and have confirmed a critical locus of 3.5 Mb by haplotype analysis in 12/13 families analyzed. We have identified five missense mutations occurring in a critical domain within the VCP gene (Valosin Containing Protein) in 10/13 families. VCP, is widely expressed, and encodes a 806 amino acid protein. It has two AAA ATPase domains, and has been implicated in two distinct and crucial cell pathways, namely membrane biogenesis and targeted protein degradation. Previous studies have shown that a ATPase D2domain mutant induced cytoplasmic vacuoles and accumulation of polyubiquitinated protein in the nuclear and membrane fractions in neurons, these findings resembling the rimmed vacuolar muscle fibers seen in this disease. It is hoped that identification of the gene for this complex disorder will help in understanding the pathogenetic mechanisms and developing new therapeutic target.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Natural history of glycine encephalopathy in 65 patients. *A. Hamosh[1], J. Hoover-Fong[1], S. Shah[1], J. Van Hove[2], J.R. Toone[3], D.A. Applegarth[3].* 1) Inst Genet Med, Johns Hopkins Univ Sch Med, Baltimore, MD, US; 2) Univ Hospital Gasthuisberg, Leuven, Belgium; 3) Univ British Columbia, Vancouver, BC, Canada.

Glycine encephalopathy results from a defect in one of the 4 peptides of the glycine cleavage system. Symptoms derive from the neurotransmitter effects of glycine at both inhibitory and excitatory sites in the CNS. To determine the natural history in a diverse population, a survey was mailed to 170 households in the International NKH Family Network; data from 6 patients from Johns Hopkins Genetics Clinic were also included. Informed consent was obtained. Data for 65 NKH patients were collected: 36 males and 29 females; 8 females died in the neonatal period, 14 died thereafter (12 M, 2 F), thus 43 survive. The mean (range) age of death was 4.14yr (2.3mo-5.5yr) for males and 0.5yr (5d-2.75yr) for females. Mean gestation was 40 wks. Mean birth weight, length, and OFC were normal. While 21/64 mothers had 1miscarriage, only 21/65 had pregnancy complications with the NKH fetus. Prenatal triple screen suggested Down syndrome in 2 (HCG,AFP,estriol). In utero hiccups were reported in 31/60; 40% once/day. At birth, 34.3% had an effective suck; 13/57 breast- and 25/58 bottlefed. Through DOL 30, 52/63 required gavage feeding, 20/52 received a G-tube, 40/63 were ventilated, 5/62 had hydrocephalus, 39/62 had abnormal eye movements and 50/52 had abnormal EEGs: burst suppression (17), focal spikes (4), hypsarrhythmia (2), other (30). Newborn brain MRI or CT were done on 34: 16 (47%) were normal, 7 (21%) showed thin/absent corpus callosum. Seizures began on day-of-life (DOL) 1-3 in 29, DOL 4-10 in 7, DOL 11-30 in 4, between 1-6 months in 11, 6mo in 2; 3 never developed seizures. Dextromethorphan (DM) and/or benzoate decreased seizures in 9/19 newborns and 14/29 30 day olds. DM and/or benzoate increased alertness in 11/23 newborns and 15/45 30day olds. Development: 38/50 smile, 16/49 sit alone, 13/40 walk, 10/50 say words, 8/49 sign words. Ten walk and say/sign words; all are male. CSF and plasma glycine levels at or near diagnosis (n=12): mean ratio was 0.2 (range 0.1-0.34). The improved survival and developmental progress of males compared with females is remarkable.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 186 from 2003 ASHG Annual Meeting

G_{M1}-gangliosidemediated ER stress response causes neuronal death in G_{M1}-gangliosidosis. *A. Tessitore[1], M. del P. Martin[1], L. Mann[1], A. Ingrassia[1], R. Sano[1], R. Proia[2], Y. Ma[1], L.M. Hendershot[1], A. d'Azzo[1].* 1) Genetics & Tumor Cell Biology, St Jude Child Res Hosp, Memphis, TN; 2) Genetics of Development and Disease Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA.

Beta-galactosidase (*-gal*) mediates the degradation of G_{M1}-ganglioside, a major component of neuronal membranes that, among other functions, modulates Ca^{2+} homeostasis. Mutations in *-gal* result in the lysosomal storage disease (LSD) G_{M1}-gangliosidosis. Children with this LSD suffer mental retardation, progressive neurodegeneration, and early death. The primary biochemical and pathologic features of G_{M1}-gangliosidosis are the storage of G_{M1}-ganglioside and the formation of membranous cytoplasmic bodies in neurons. The pathogenic mechanisms underlying neurodegeneration in this disease are unknown. We have investigated whether the endoplasmic reticulum (ER)-stress response contributes to the neuronal apoptosis that characterizes the CNS disease of the murine model of G_{M1}-gangliosidosis, and likely of patients. In spinal neurons of *-gal*$^{-/-}$ mice, abnormal induction of CHOP and JNK accompanied the induction of ER stress-specific caspase-12. This phenotype was rescued in mice double deficient for -gal and -4-N-acetylgalatosaminyltransferase (GalNAcT), which lacks G_{M1}-ganglioside. In addition, G_{M1} loading of wild type mouse embryonic fibroblasts (MEFs) recapitulates the morphologic changes of *-gal*$^{-/-}$ MEFs and results in activation of the ER stress pathway and cell death. Our findings suggest that accumulation of G_{M1}-ganglioside activates the ER stress response, which ultimately provokes sporadic apoptosis and progressive neurodegeneration. These findings point to a novel mechanism of neuronal apoptosis mediated by ER stress, which results from intracellular accumulation of a glycolipid rather than unfolded proteins.These studies were supported by the National Institutes of Health grant DK52025.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 187 from 2003 ASHG Annual Meeting

Roles of synergistic heterozygosity and dietary fat in metabolic crises of acyl-CoA dehydrogenase deficiencies in mouse models. *P.A. Wood[1], A.M. Schuler[1], J. Vockley[2], D. Matern[2], P. Rinaldo[2].* 1) Dept Genetics, Univ Alabama at Birmingham, Birmingham, AL; 2) Dept. Medical Genetics, Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.

We have used mice with acyl-CoA dehydrogenase deficiencies to better understand the pathogenesis and dietary management of children with inborn errors of mitochondrial fatty acid -oxidation. Mice double heterozygous for deficiencies of very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency, long-chain acyl-CoA dehydrogenase (LCAD) deficiency, and short-chain acyl-CoA dehydrogenase deficiency (SCAD) develop disease when challenged by cold (4C). Ability to thermoregulate is a reproducible measure of metabolic competence. There was severe hypothermia found in VLCAD+/-//LCAD+/- mice and LCAD+/-//SCAD+/- mice; whereas, single heterozygous mice, e.g., VLCAD+/-, or wild-type mice maintained a normal temperature. Biochemical changes were significantly milder in double heterozygotes as compared to homozygous deficient mice. Thus, disease occurred in double heterozygous mice. We also found that feeding a high-fat diet consisting of medium-chain triglycerides (MCT) is beneficial to LCAD-/- mice only when fed throughout their gestation, nursing, and weaning. Survival increased to 93% from 50% for long-chain triglyceride (LCT) diet fed LCAD-/- mice; whereas weaning LCAD-/- mice onto an MCT diet was of no benefit, percent survival was 50% and 57% respectively. In contrast, feeding LCT diet to SCAD-/- mice at weaning increased survival to 94% from 40% when fed a MCT diet. In summary, dietary fat manipulations had profound beneficial effects as far as resistance to metabolic challenge. In LCAD deficiency this required feeding an MCT diet to the gestating/nursing mother with subsequent weaning of the pups onto this diet (P=0.039). In contrast, we found a significant increased survival (P=0.0003) in SCAD-/- mice fed an LCT diet at weaning. These results demonstrate that in some disorders high dietary fat may be beneficial, but there may be major differences in these dietary influences among the different inborn errors of fatty acid oxidation as far as what stage of life the fat is presented.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 188 from 2003 ASHG Annual Meeting

***Abcd3-/-* mice have a non-shivering thermogenesis defect related to a disturbance in fasting fuel homeostasis.** *I. Silva-Zolezzi*[1,2]*, K.J. Hebron*[1]*, S.J. Mihalik*[3]*, G. Thomas*[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, IPN, Mexico; 3) Kennedy Krieger Institute, Baltimore, MD.

The known human peroxisomal half ABC transporters are: ALD, ALDR, PMP70 and P70R, encoded by *ABCD1, 2, 3 and 4*, respectively. Mutations in *ABCD1* cause X-linked Adrenoleukodystrophy. Disease association of the other is unknown. We produced *Abcd3-/-* mice which are viable, have enlarged peroxisomes; reduced hepatic glycogen; dicarboxylic aciduria; defective phytanic and pristanic oxidation; and defective non shivering thermogenesis (DNST) (drop in body temp to <15C after 6h at 5C), similar to mice with defects in mitochondrial -oxidation (*Acadl-/-*). We postulate that PMP70 contributes to the transporter of -branched fatty acids (BFA) into peroxisomes. In its absence, BFA accumulate leading to inappropriate activation of PPAR (fasting fuel homeostasis). This is supported by over expression of PPAR target genes (*Acadm, Aox, Acaa1, Fabpl and Cyp4a10*) in *Abcd3-/-* liver. FA mobilization and lipid content in brown fat (BAT) is similar in *Abcd3-/-* and controls, but *Acadl-/-* mice accumulate lipid. In *Abcd3-/-* mice, BAT expression of PPAR target genes, including UCP-1, was as controls. Blood glucose levels in this mice are ~50% of controls after 4.5 h at 5C, similar to *Acadl-/-* mice. Cold tolerance was partially corrected in *Abcd3-/-* mice after 10 d on a high carb diet or carnitine supplementation (0.5g/Kg/d) for 4 wks. To further analyze the mechanisms underlying this phenotype we performed an expression analysis in *Abcd3-/-* and control livers with Affymetrix microarrays (U74A). Initial results confirmed over expression of PPAR target genes including those analyzed by Northern blot. We observed changes in 285 genes, 129 showed at least a 2X increase and 156 a 2X decrease over controls. Preliminary analysis indicate expression changes in genes of FA metabolism, regulation of cell cycle and inflammation. These results support our hypothesis that the DNST in *Abcd3-/-* mice is associated with abnormal fasting fuel homeostasis.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 189 from 2003 ASHG Annual Meeting

Mouse models of X-Linked adrenoleukodystrophy: overlapping function of ABCD1 and ABCD2 transporters and implications for therapy. *A. Pujol[1], C. Camps[1,2], C. Hindelang[1], M. Giros[2], T. Pampols[2], I. Ferrer[3], J.L. Mandel[1]*. 1) Dept Human Molecular Genetics, IGBMC, Strasbourg, France; 2) Institut de Bioquimica Clinica,Bercelona, Spain; 3) Institut de Neuropatologia, Hospital Universitary de Bellvitge, Barcelona, Spain.

X-linked adrenoleukodystrophy is a severe neurological disorder presenting with central or peripheral demyelination and impaired function of adrenals. X-ALD patients accumulate very long chain fatty acids (VLCFA) in plasma and tissues, notably in the adrenal cortex and nervous system. This disease is characterised by extensive phenotypic variability, even among patients sharing the same mutation. The two main neurological phenotypes are the severe childhood cerebral form and the slowly progressive adult adrenomyeloneuropathy. A mouse model of the disease also accumulates VLCFAs in target organs, and has recently been shown to develop an adrenomyeloneuropathy-like phenotype (Pujol et al, Hum Mol Genet 2002).The gene mutated in the disease (ABCD1) codes for a peroxisomal ABC transporter protein (ALDP). Its closest homolog (ABCD2, 88% similarity with ALDP) codes for another peroxisomal transporter (ALDRP), that sits at the crossroad of SREBP and PPAR receptor signalling. To investigate the issue of functional redundancy between the two transporters, we have generated mice deficient for ALDRP and mice overexpressing ALDRP. Stable overexpression of the ALDRP by transgenesis in the ALD-deficient mouse background leads to full correction of VLCFAs target organs, correlating with an improvement of the neurological AMN-like phenotype in mice, a fact that turns ABCD2 into a prime target for pharmacogenomics approaches to X-ALD treatment.Interestingly, ALDRP KO animals exhibit a late-onset spinocerebellar ataxia, making of this gene a candidate for similar human syndromes at 12q12. Double mutants ALDP/ALDRP exhibit an accelerated neurological phenotype with signs of inflammation in CNS, in absence of a more severe accumulation of VLCFAs in tissues or plasma. Thus, double mutants are a valuable model for the study peroxisomal physiopathogenesis and for evaluation of therapies.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 190 from 2003 ASHG Annual Meeting

Development, characterization, and treatment of a hypomorphic SLOS mouse model. *L.S. Correa-Cerro[1], C.A. Wassif[1], L. Kratz[2], R.I. Kelly[2], F.D. Porter[1].* 1) NICHD/NIH, Bethesda, MD; 2) The Kennedy Krieger Institute, Baltimore, MD.

The Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive, malformation syndrome due to mutation of the 7-dehydrocholesterol reductase gene (*DHCR7*). DHCR7 reduces 7-dehydrocholesterol (7-DHC) to cholesterol. SLOS patients typically have decreased cholesterol and increased 7-DHC levels. Dietary cholesterol supplementation has been used to treat SLOS patients and simvastatin therapy to decrease 7-DHC levels has been proposed. The most common missense mutation in *DHCR7* is 278C>T (T93M). Mice homozygous for a null disruption () of *Dhcr7* die soon after birth. To investigate therapeutic interventions for SLOS, we generated a hypomorphic SLOS mouse model by "knocking in" a T93M mutation using targeted homologous recombination in embryonic stem cells. T93M/T93M mice were phenotypically normal; however, T93M/ mice had 2-3 toe syndactyly. 2-3 toe syndactyly is the most common physical finding in SLOS patients. Sterol profiles of liver, cortex, midbrain, and kidney in both one day old and six weeks old mice showed elevated 7-DHC. As expected, 7-DHC levels were higher in the T93M/ compared to the T93M/T93M mice. To experimentally evaluate dietary cholesterol supplementation we compared T93M/ mice on a regular versus cholesterol suplemented diet. After 8 months, no survival or pathological differences were found. Sterol analysis of tissues showed biochemical improvement in some peripheral tissues. As expected, cortex sterol levels were not changed. Neuromuscular testing (vertical pole and hanging wire) indicated that cholesterol supplementation may improve neuromuscular status. Treatment of T93M/ mice with simvastatin (10 mg/kg/d) for three weeks significantly decreased 7-DHC levels in some peripheral tissues. Notably, simvastatin therapy also decreased dehydrocholesterol levels in cortex tissue. Correcting the sterol abnormalities in the central nervous system may positively impact behavioral and developmental problems found in SLOS patients. In conclusion, we are describing for the first time the development of a viable SLOS mouse model, and have demonstrated a beneficial effect of both cholesterol and simvastatin therapy in this mouse model.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 191 from 2003 ASHG Annual Meeting

Understanding Hermansky-Pudlak syndrome by visualizing intracellular trafficking patterns. *M. Huizing, H. Dorward, A. Helip-Wooley, W.A. Gahl.* NHGRI,NIH,Bethesda,MD.

In Hermansky Pudlak syndrome (HPS), defects in melanosomes, platelet dense bodies and lysosomes cause albinism, prolonged bleeding and intracellular storage of lipid-protein complexes. We identified the molecular defects in 115 patients with HPS due to one of the six known HPS-causing genes. One of these genes, *ADTB3A*, codes for a subunit of adaptor complex-3 (AP-3), a coat protein involved in vesicle formation. Using *ADTB3A* deficient melanocytes, we previously showed that tyrosinase remains in the peri-nuclear region while tyrosinase-related protein (TYRP1) migrates with melanosomes to the dendrites. Thus, AP-3 recognizes tyrosinase, but not TYRP1, and these two proteins travel to melanosomes by different routes. Based upon the power of cell imaging in dissecting these vesicular trafficking pathways, we extended our studies to HPS-1, HPS-3 and HPS-4 fibroblasts. Each of these cell types is deficient in an HPS-causing gene coding for a protein of unknown function. Using fluorescent live cell imaging, we recorded: 1. Intracellular localization of Green Fluorescent Protein (GFP)-tagged HPS proteins; 2. Formation and trafficking of GFP-tagged lysosomal markers LAMP1 or CD63 in affected cells; 3. Fluid phase endocytosis and lysosomal degradation (Rh-labeled albumin) in affected cells. HPS1, HPS3 and HPS4 all localized in the perinuclear area, and HPS3 also localized to dendrite tips in melanocytes. LAMP1 and CD63 accumulated in large membranous structures in HPS-1 and HPS-4 cells, while HPS-3 cells showed a normal lysosomal distribution. HPS-3 cells showed a normal albumin uptake and lysosomal degradation, while HPS-1 and HPS-4 cells accumulated albumin in the perinuclear area, with minimal transport to the lysosome. Normal albumin distribution was restored in HPS1 deficient cells by overexpressing GFP-HPS1. These are the first studies to visualize the effects of genetic mutations on vesicular trafficking in human HPS cells. Such imaging studies indicate the intracellular location of HPS gene products, provide insight into functions and interactions of HPS gene products, and suggest therapeutic maneuvers, which can be tested in cultured HPS fibroblasts.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 192 from 2003 ASHG Annual Meeting

A Phase I/II Randomized, Double Blind, Two Dose Group Study of Recombinant Human N-Acetylgalactosamine-4-Sulfatase (rhASB) Enzyme Replacement Therapy in Patients with Mucopolysaccharidosis VI (Maroteaux-Lamy Syndrome): 96 Week Progress Report.

J. Hopwood[1], C. Whitley[2], L. Waber[3], R. Pais[4], R. Steiner[5], B. Plecko[6], P. Kaplan[7], J. Simon[8], J. Waterson[8], P. Harmatz[8]. 1) Women's & Children's Hosp, N Adelaide, Australia; 2) U Minnesota Med School; 3) U Texas SW Med Center; 4) E Tennessee Children's Hosp; 5) Oregon Health & Science U; 6) U Kinderklinik Graz, Austria; 7) Children's Hosp Philadelphia; 8) Children's Hosp & Res Ctr Oakland.

Mucopolysaccharidosis VI (MPS VI; Maroteaux-Lamy syndrome) is a lysosomal storage disease caused by a deficiency of the enzyme N-acetylgalactosamine-4-sulfatase (ASB), leading to a progressive disorder with multiple organ and tissue involvement. A randomized, two-dose, double-blind study to evaluate safety and efficacy of weekly treatment with rhASB in MPS VI patients was completed, followed by an open-label extension. Initially, patients were randomized to 24 weeks of either high (1.0 mg/kg) or low (0.2 mg/kg) doses of rhASB. The 2 patients on low dose were advanced to the 1 mg/kg dose after the 48 week evaluations. Six patients completed at least 24 weeks of treatment (3 Males; age 7-16 yrs); 5 patients have completed at least 96 weeks. One patient withdrew from the study for reasons unrelated to study drug. There were 16 SAEs, 1 drug-related. One patient developed cutaneous reactions during the 2nd year of treatment that are managed by increasing infusion time and dose of antihistamine pretreatment. Two patients developed high antibody titers during the study that decreased to baseline levels by week 96. Reductions in total urinary GAG excretion were maintained at 96 weeks. The rollover to the higher dose resulted in an additional decline in urinary GAG. A mean percent increase of 95.75 105.59 in the 6 Minute Walk Test and 13.75 3.77 degrees in shoulder flexion were observed at week 96. All 4 patients tested at 96 weeks had improvement in forced vital capacity. In conclusion, treatment after two years remained well-tolerated with minimal side effects, improvements in endurance were maintained and antibody titers were low. (Sponsored by BioMarin Pharmaceutical Inc., Novato).

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 193 from 2003 ASHG Annual Meeting

Novel neurometabolic condition responsive to folinic acid supplementation. *P. Moretti*[1], *K. Hyland*[2], *T. Bottiglieri*[2], *J. Neul*[3], *G. Miller*[3], *F. Scaglia*[1]. 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Institute of Metabolic Disease, Dallas, TX; 3) Dept of Pediatrics, Houston, TX.

Two distinct transport systems of folates across mammalian cell membranes have been described. The first system is represented by the reduced folate carrier (RFC), which mediates a low affinity, high-capacity system for the uptake of reduced folates at high (M) concentrations. A defect in this transporter may lead to hereditary folate malabsorption (MIM 229050). The second system, a family of membrane-associated folate receptors (FR) mediate a high affinity, low capacity system and operate at low (nM) concentrations. The folate receptor-1 (FR-1) protein is localized at the basolateral surface of the choroids plexus, and has a high binding affinity for 5-methyltetrahydrofolate (5-MTHF). A disorder of folate transport affecting this system was recently described among five children who presented at age six months with a slowly progressive neurological disease characterized by psychomotor retardation, cerebellar ataxia, dyskinesia, pyramidal signs, and seizures. This condition was accompanied with low levels of 5-MTHF in cerebrospinal fluid (CSF) and was responsive to folinic acid supplementation. The molecular etiology of disturbed folate transfer across the blood-brain barrier remains unknown. Here we report an additional patient with this novel condition. The proband is a five-year-old girl who presented at age 6 months with a progressive neurological condition that included developmental delay, regression of motor skills and language, loss of purposeful hand movement, cerebellar ataxia, dyskinesia, pyramidal signs, and occasional seizures. Analysis of CSF revealed low values for 5-MTHF. A significant elevation of CSF homocysteine was noted. Total plasma homocysteine was normal. Oral treatment with folinic acid resulted in significant clinical amelioration. Preliminary results of an ongoing work-up suggest a defective folate transfer across the blood-brain barrier. To further characterize this condition, analysis of the FR genes and FR protein function as well as regulatory processes of folate receptor expression and folate transfer mechanism will be required.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 194 from 2003 ASHG Annual Meeting

The multiple sulfatase deficiency gene, *SUMF1*, and its therapeutic potential for sulfatase deficiencies. *A. Ballabio*[1], *M.P. Cosma*[1], *S. Pepe*[1], *I. Annunziata*[1], *C. Settembre*[1], *M. Grompe*[3], *A. Biffi*[4], *L. Naldini*[4], *G. Parenti*[2]. 1) Telethon Institute of Genetics and Medicine, Naples; 2) Federico II University, Naples; 3) OHSU, Portland, Oregon; 4) Telethon Institute of Gene Therapy, Milan.

In multiple sulfatase deficiency (MSD), the enzymatic activities of all sulfatases are profoundly impaired due to a defect in a post-translational modification mechanism. Using microcell mediated chromosome transfer we identified the MSD gene, *SUMF1* (SUlfatase Modifying Factor 1). Simultaneous overexpression of *SUMF1* and human sulfatase cDNAs resulted in a strikingly synergistic increase, up to 50 fold, of the enzymatic activity of each sulfatase examined. Each of the missense mutations identified in MSD patients were also tested and showed loss of function of variable degree. A much weaker synergistic effect was observed using *SUMF2*. Towards the goal of achieving an efficient production of active sulfatases for enzyme replacement in Hunter and Morquio A syndromes, the *SUMF1* gene was transiently expressed in two different cell lines (developed for mass enzyme production by Transkaryotic Therapies, Inc.) that stably overproduce either iduronate-2-sulfatase (I2S) or N-acetyl-galactosamine-6-sulfatase (GALNS). A significant increase of sulfatase activity was observed for both enzymes. The spectrum of activity of SUMF1 on all the 13 human sulfatases is being characterized. Finally, using lentiviral-mediated gene delivery we are studying the enhancing effect of SUMF1 on arylsulfatase A (ARSA) in a murine model of metachromatic leukodystrophy. Preliminary data obtained in ex vivo bone marrow cells from these mice indicate a strong enhancing effect of SUMF1 on sulfatase activity. We are in the process of evaluating the therapeutic potential of this enhancing effect after transplantation of the transduced cells back into mice. Delivery of sulfatase cDNAs will also be performed on a transgenic mouse overexpressing the *SUMF1* gene. These data will have important implications on the feasibility of enzyme replacement and gene therapy on eight diseases due to sulfatase deficiencies.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 195 from 2003 ASHG Annual Meeting

Genome anatomy of Wilms' Tumor: genome-wide LOH analysis reveals unique signatures and tumor subgroups.

R. Krahe[1,2], *B.W. Hendrickson*[1,2], *S. Colella*[1,2], *R. Alam*[1], *F.A. Wright*[3], *V. Huff*[1]. 1) Sect Cancer Genetics, Univ Texas MD Anderson CA Ctr, Houston, TX; 2) Human Cancer Genetics Program, Comp. Cancer Center, Ohio State Univ., Columbus, OH; 3) Dept. of Biostatistics, Univ. of North Carolina, Chapel Hill, NC.

Wilms' tumor (WT) of the kidney is the most common pediatric solid tumor. WT is genetically heterogeneous, involving multiple genetic and epigenetic alterations, with approximately 15% of cases having *WT1* mutations. Loss-of-heterozygosity (LOH) is an established method of searching for tumor suppressor genes (TSGs). To identify novel WT TSGs, we performed an unbiased genome-wide LOH scan with 400 microsatellite markers in 94 matched normal/tumor pairs (21 with *WT1* mutations, 19 sporadic and 2 familial; and 73 without *WT1* mutations, 63 sporadic and 10 familial) using semi-automated, fluorescent capillary allelotyping. After establishing a WT-specific LOH ratio threshold (0.45 vs. 0.67), we confirmed high rates of LOH in the established WT regions (*WT1* in 11p13 and *WT2* in 11p15.5) and identified several other high LOH regions, harboring candidate TSGs, as well as several regions and entire chromosomes with extremely low LOH, harboring protective or essential genes. Using a likelihood-based approach that provides a LOD-like score to identify regions of LOH, we confirmed the high LOH regions. Although low LOH ratios were a genome-wide phenomenon, sample-specific examination revealed unique, low-ratio tumor signatures that spanned very few markers and chromosomes for any given sample. This suggests that relatively few genetic events underlie WT tumorigenesis. By cluster analysis we identified several multi-marker and multi-chromosomal LOH interactions. The most prominent delineation was between samples with and without *WT1* mutations. Interestingly, for samples without *WT1* mutations both familial and sporadic cases clustered together. The complex yet unique, genome-wide patterns of LOH ratios for a given sample provide a glimpse into the natural history of the events underlying an individual tumor. Taken together, these data provide evidence for separate modes of tumorigenesis depending on the mutation status for *WT1*.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 196 from 2003 ASHG Annual Meeting

Six genes identified by comparative microarray analysis distinguish papillary from follicular thyroid carcinomas.

M.A. Aldred[1], Y. Huang[1], S. Liyanarachchi[1], N.S. Pellegata[1], O. Gimm[2], S. Jhiang[1], R. Davuluri[1], A. de la Chapelle[1], C. Eng[1]. 1) The Ohio State University, Columbus, OH; 2) Martin-Luther-University, Halle-Wittenburg, Germany.

Thyroid carcinoma is collectively the most common of the endocrine cancers but the molecular mechanisms by which the different types of thyroid carcinoma develop are not well understood. We have previously conducted independent microarray expression analyses of the two most common types of non-medullary thyroid carcinoma, namely papillary (PTC) and follicular thyroid carcinomas (FTC). In this study, we sought to combine our datasets to shed light on the similarities and the differences between these tumor types. Bioinformatics analysis was performed to normalize the two datasets and remove inter-laboratory variability. PTCs show distinct groups of genes that are up- or down-regulated respectively compared to normal thyroid, whereas the predominant changes in FTCs are downregulation. A subset of these genes was downregulated in both tumor types, suggesting some commonality, but the most intriguing finding was a small group of genes that show upregulation in PTCs but downregulation in FTCs, when compared to normal thyroid. Six genes could collectively distinguish the two tumor types: PTCs were upregulated for at least two of *CITED1*, *ARHI*, *claudin-10* and *IGFBP6* but showed no change in expression of *caveolin-1* or *-2*; FTCs conversely did not express *claudin-10* and were downregulated for at least two of *ARHI*, *IGFBP6* and *caveolin-1* or *-2*. Our results suggest that PTC and FTC either have different molecular origins or diverge distinctly from a common origin. Furthermore, if verified in a larger series of tumors, these genes could, in combination with known tumor-specific chromosome translocations, form the basis of a valuable diagnostic tool.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 197 from 2003 ASHG Annual Meeting

The neurofibromatosis 2 (NF2) tumor suppressor schwannomin interacting protein HRS regulates EGF receptor signaling and trafficking in schwannoma cells. *D.R. Scoles*[1,2], *D.H. Gutmann*[4], *S.M. Pulst*[1,2,3]. 1) Burns and Allen Research Institute and Division of Neurology, Cedars-Sinai Medical Center, Los Angeles, CA; 2) School of Medicine, University of California Los Angeles, Los Angeles, CA; 3) Department of Neurobiology, University of California Los Angeles, Los Angeles, CA; 4) Department of Neurology, Washington University School of Medicine, St. Louis, MO.

The hepatocyte growth factor receptor tyrosine kinase substrate HRS is a vesicular protein that is important for the trafficking of receptor tyrosine kinases (RTKs) to the lysosome where RTKs are degraded. Previously we identified HRS as an interactor to the neurofibromatosis 2 (NF2) tumor suppressor schwannomin. Mutation in the NF2 gene is one of the most common causes of benign brain tumors including schwannomas and meningiomas. We hypothesize that schwannomin stimulates HRS-mediated trafficking of growth-activating RTKs to the lysosome. To determine a role for schwannomin in HRS-mediated trafficking of RTKs, we have developed doxycycline-inducible HRS and schwannomin cell line model systems using RT4 schwannoma cells that also constitutively express epidermal growth factor receptor tagged with green fluorescent protein. Expression of HRS in RT4 cells results in relocalisation of EGFR to perinuclear structures that label with lysosomal marker antibodies. Pulse-chase studies indicated that HRS expression resulted in increased degradation of active (EGF-bound) receptor. In RT4 cells, EGF treatment strongly induces Stat3 phosphorylation. This Stat3 phosphorylation was inhibited by HRS. In conclusion, HRS is a powerful regulator of EGFR signaling in RT4 schwannoma cells. Our model system will be useful to refine the role for schwannomin in HRS-mediated inhibition of EGFR signaling. This research was supported by Department of Defense grant DAMD17-00-1-0553 to DRS, grants RO1 NS37883 to SMP, and the Carmen and Louis Warschaw endowment for neurology.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

The NF2 tumor suppressor schwannomin interacts with the eukaryotic initiation factor 3 (eIF3) subunit p110. *Y. Qin[1], S. Pulst[1,2,3], D.R. Scoles[1,2]*. 1) Burns and Allen Research Institute and Division of Neurology, Cedars-Sinai Medical Center, Los Angeles, CA; 2) School of Medicine, University of California Los Angeles, Los Angeles, CA; 3) Department of Neurobiology, UCLA School of Medicine, Los Angeles, CA.

Mutation in the neurofibromatosis 2 (NF2) tumor suppressor gene is one of the most common causes of benign brain tumors including schwannomas and meningiomas. To identify interactors of the NF2 protein schwannomin, we used the Gal4-based yeast two-hybrid method to screen a human lymphocyte library. Among three putative interactors, we successfully validated one, the eukaryotic initiation factor 3 (eIF3) subunit p110, as a schwannomin interacting protein. Using the yeast two-hybrid system, we showed p110 binds the FERM domain of schwannomin. We verified the interaction between schwannomin and full-length p110 by using the non-transcriptionally-based ras-rescue yeast two-hybrid system. We also validated that schwannomin binds p110 directly in vitro, and that schwannomin and p110 co-immunoprecipitate from and co-localize in STS26T schwannoma cells. By yeast two-hybrid testing, p110 does not bind schwannomin containing the NF2 missense mutations L46R, K364I, L535P, and Q538P. The eIF3 protein is required for the 40S ribosomal subunit bound to the ternary complex (eIF2-GTP-methionine) to interact with the 5' end of the mRNA and is essential for the initiation of translation. Our data strongly support a direct interaction between schwannomin and p110 in cells relevant to the NF2 phenotype. This interaction is abolished by disease-causing NF2 missense mutations. Because p110 is tied to proliferation in some model systems, we hypothesize that schwannomin's interaction with p110 serves to reduce p110's proliferative properties mediated through protein translation. This research was supported by Department of Defense grant DAMD17-00-1-0553 to DRS, grant RO1 NS37883 to SMP, and the Carmen and Louis Warschaw endowment for neurology.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Targeted disruption of mouse suppressor of fused, a negative regulator of hedgehog signaling. *A.F. Cooper, K.P. Yu, M. Brueckner, J.M. McGrath, A.E. Bale.* Yale Univ, New Haven, CT.

The Hedgehog (Hh) signaling pathway is a key regulator of embryogenesis and continues to regulate cell growth and differentiation in adult tissues. Mutations in patched (PTCH), a negative regulator of the pathway, cause constitutive activation of Hh signaling in almost all basal cell carcinomas, a substantial fraction of medulloblastomas, and in some rhabdomyosarcomas and other tumors. Suppressor of fused (Sufu), another negative regulator of Hh signaling, antagonizes the activity of the GLI transcription factors that transduce the Hh signal. Analogous to PTCH, inactivating mutations of human Sufu have been detected in medulloblastomas. To study the role of Sufu in tumorigenesis and development, we constructed a knockout mouse by targeted disruption of exon 7 of the Sufu gene. Heterozygotes were viable, morphologically normal, and equal in size to their wild type littermates. To evaluate cancer predisposition in Sufu heterozygotes (129SV/B6 background), we used 4 Gy of ionizing radiation to induce neoplasia. Necropsy after 12 months showed no excess of cancer compared with irradiated isogenic controls. Interbreeding of heterozygotes produced no live-born homozygous offspring, and timed matings showed that Sufu -/- mice die between E9.0 and E10.5. The gross phenotype, which appears at E9.5, is an open neural tube. The Hh pathway plays an essential role in dorsal-ventral patterning of the neural tube. Immunohistochemistry with region-specific markers of neural progenitor cells indicated excess Hh effect and dorsalization of cell types. The Sufu -/- embryos also exhibited randomized heart looping. Pitx2, an inducer of heart laterality normally expressed in the left lateral mesoderm, was seen bilaterally in E8.5 Sufu -/- embryos. These studies provide evidence for an essential role of Sufu in vertebrate development. Sufu -/- mice exhibited CNS and cardiac defects similar to those seen in other Hh pathway mutants, but did not have polydactyly or other digit malformations which are common with disruption of this pathway. These mice appear to be less cancer prone than Ptch heterozygotes.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 200 from 2003 ASHG Annual Meeting

Deletion of the Carney Complex Gene PRKAR1A leads to immortalization of primary mouse embryonic fibroblast cells. *L. Kirschner[1], A. Grinberg[2], H. Westphal[2], C.A. Stratakis[3].* 1) Dept Human Cancer Genetics, Ohio State Univ, Columbus, OH; 2) LMGD, NICHD, NIH, Bethesda, MD; 3) SGEN, NICHD, NIH, Bethesda, MD.

Protein Kinase A (PKA, cyclic-AMP dependent protein kinase), is a key regulator of endocrine signaling processes, and exists in cells as a heterotetramer composed of 2 catalytic and 2 regulatory subunits. *PRKAR1A*, the gene coding for the Type 1A regulatory subunit, is ubiquitously expressed in humans and appears to be the most highly expressed of the regulatory subunits. Inactivating mutations of this gene cause the autosomal dominant tumor syndrome Carney Complex, which is characterized by spotty skin pigmentation, myxomas, schwannomas, and endocrine tumors. In order to understand the mechanism by which loss of this gene leads to tumorigenesis, primary mouse embryonic fibroblasts (MEFs) homozygous for a conditional null allele of *Prkar1a* were generated. Treatment of these cells with cre recombinase led to the generation of *Prkar1a* null cells, as evidenced by the absence of the protein on Western blotting. At the cellular level, primary MEFs treated with a control vector underwent normal cellular senescence after 10-14 passages, whereas cells treated with cre were immortalized. Phenotypically, the cre-treated cells demonstrated a morphological change, in which they lost the elongated appearance of fibroblasts and became polygonal. Measurement of PKA subunits by Western blotting demonstrated a lack of down-regulation of the PKA catalytic subunit (PKAC) and only minimal up-regulation of other PKA regulatory subunits (R1B, R2A, R2B). Although MEFs lacking Prkar1a were immortal, they were not transformed, as evidenced by retention of contact inhibition. Introduction of an activated form of the *ras* oncogene into the *Prkar1a* null cells led to cellular transformation and a loss of contact inhibition. The mechanisms underlying these observations are currently under investigation. Understanding these processes in a well-defined tissue culture model should help provide insights into the mechanism of tumor formation in patients with this inherited tumor syndrome.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 201 from 2003 ASHG Annual Meeting

A Transgenic Mouse Model for Studies of Cyclic-AMP-dependent PKA Function. *K.J. Griffin[1], L. Kirschner[2], S. Stergiopoulos[1], S. Lenherr[1], E. Claflin[1], L. Matyakhina[1], A. Bauer[3], J.A. Carney[4], C.A. Stratakis[1].* 1) SEGEN, DEB, NICHD, NIH, Bethesda, MD; 2) Department of Medicine, Ohio State University, Columbus, OH; 3) WRAMC, Washington, DC; 4) Emeritus Staff, Mayo Clinic, Rochester, MN.

Carney complex (CNC) is a multiple neoplasia syndrome consisting of endocrine tumors (adrenal, pituitary, thyroid, and gonads), schwannomas, and myxomas. Half of CNC patients have mutations in *PRKAR1A*, the regulatory I subunit of protein kinase A (PKA) which increase cAMP-stimulated kinase activity in tumors. To circumvent the embryonic lethality of the *prkar1a -/-* mouse, we generated a transgenic mouse expressing an antisense construct for exon 2 of *PRKAR1A(X2AS)* under the control of a conditional promoter. Tissues from *X2AS* mice mimic the biochemical changes seen in CNC tumors. Message levels and protein expression *PRKAR1A* are decreased up to 70%. The pattern of cAMP-stimulated PKA activity is similar to that seen in CNC: activites in *X2AS* mouse tissues were 1.4-1.8 times higher than controls ($p < 0.05$). Fourteen *X2AS* mice, but no control mice, have developed generalized tonic/clonic seizures; 23 exhibit abnormal posturing. The *X2AS*-expressing mice are lean and have lower body weights than control mice. Of 5 *X2AS* and 7 control mice necropsied at ~1 year of age, 1 *X2AS* and 3 control mice developed histiosarcoma; 2 *X2AS* mice had lymphoma. Three *X2AS* mice had follicular hyperplasia of the thyroid; one developed bilateral follicular adenomas. Four *X2AS* mice have had hip swelling from inguinal hernias. Histologically, almost all *X2AS* mice showed histiocytic hyperplasia in lymph nodes, spleen, and liver. Subunit staining in *X2AS* mice was altered in multiple tissues, with a decrease in immunostaining for RI and a corresponding increase in RII. Although the *X2AS* mice have not developed many tumors typical of CNC by 12 months, they appear to exhibit abnormalities consistent with down-regulation of the *PRKAR1A* gene. Continuing observation of these animals and further studies may provide insight into the mechanisms leading to cAMP-related abnormal growth and proliferation in this syndrome.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Large scale genetic screen for novel MEN1 interactors. *V. Busygina, K. Suphapeetiporn, A.E. Bale.* Dept Genetics, Yale Univ Sch Medicine, New Haven, CT.

Multiple Endocrine Neoplasia type 1 (MEN1) is an autosomal dominant cancer syndrome affecting primarily the pituitary, parathyroids and pancreatic islets. MEN1 encodes a nuclear protein, menin, which has no homology to any other known protein and no recognized functional motifs besides nuclear localization signals. Biochemical studies in mammals suggest that menin interacts with several transcription factors including JunD, Smad3, and NF-B.
We used *Drosophila* as a model organism to analyse menin function. In previous studies we showed that loss of function of *Drosophila* MEN1 (DMEN1) leads to a mild phenotype characterized by sensitivity to DNA damage. Targeted overexpression of DMEN1 caused a split thorax, which was related to repression of Jun function in controlling thoracic closure. MEN1 was further shown to have general co-repressor activity.
In order to identify novel genetic interactors of MEN1 we performed a modifier screen in which flies with thoracic cleft phenotype related to DMEN1 overexpression were crossed individually with 2300 lines carrying independent insertions of Enhancer Promoter (EP) elements, which direct misexpression of gene when inserted at its 5' end. *Drosophila* carrying both a DMEN1 transgene and an EP element were compared with flies carrying the EP element, alone, and DMEN1, alone. Any non-additive interactions in this initial screen are being confirmed by additional genetic crosses. Consistent with our previous finding that MEN1 interacts with Jun, the current screen identified two members of JNK pathway as modifiers of the MEN1-related split thorax phenotype: Connector of kinase to AP-1 (Cka) and puckered (puc). In support of the role of MEN1 as a general transcriptional co-repressor, we identified interactors in the class of transcription factors (scalloped, lola, escargot, aop, mef2, buttonless), comodulators (nej, CtBP, emc) and chromatin remodeling genes (osa, woc, trithorax, HmgD2). The screen also identified two DNA damage response genes (ligase 4, CK1alpha), which might be mediators of the effect of MEN1 on DNA damage.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Intronic Single Nucleotide Polymorphisms in the *RET* Proto-oncogene are Associated with a Subset of Apparently Sporadic Pheochromocytoma and May Modulate Age of Onset. *S.R. McWhinney[1], G. Boru[1], P.K. Binkley[2], M. Peczkowska[3], A.A. Januszewicz[3], H.P.H. Neumann[4], C. Eng[1]*. 1) Human Cancer Genetics Prog, Ohio State Univ, Columbus; 2) Heart-Lung Research Institute, Ohio State Univ, Columbus; 3) Institute of Cardiology, Warsaw, Poland; 4) Nephrology and Hypertension, Albert-Ludwigs-University of Freiburg, Germany.

Approximately 75% of pheochromocytomas are sporadic. Germline mutations in *RET, VHL, SDHB* and *SDHD* have been shown to cause the 25% that are hereditary. Germline high penetrance gain-of-function *RET* mutations cause multiple endocrine neoplasia type 2, of which medullary thyroid carcinoma (MTC) and pheochromocytoma are components, while loss-of-function mutations cause Hirschprung disease (HSCR). A low penetrance founder locus, in linkage disequilibrium with a *RET* ancestral haplotype comprising specific alleles at three intron (IVS) 1 single nucleotide polymorphisms (SNP) [haplotype 0] and SNP A45A, predisposes to the majority of isolated HSCR. A different low penetrance locus, in linkage disequilibrium with IVS 1 haplotype 2 and SNP S836S, was associated with a subset of sporadic MTC. We, therefore, sought to determine if *RET* might also be a low penetrance gene for apparently sporadic pheochromocytoma. We analyzed 104 pheochromocytoma cases without germline mutations in *RET, VHL, SDHB* and *SDHD* for their status at A45A, S836S, three IVS 1 SNPs and a novel upstream insertion/deletion variant. Pheochromocytoma cases were not associated with either A45A or S836S, but were associated with haplotype 0 (P=0.032). However, unlike HSCR, this pheochromocytoma-associated haplotype 0 was not associated with A45A. The association was strengthened when allele frequencies from a more 5 polymorphism were taken into account (P=0.016). In addition, we found a significant correlation between individuals diagnosed under 40 years and haplotype 0. Taken together, our observations suggest the presence of a low-penetrance pheochromocytoma susceptibility locus in a region upstream of the putative loci for HSCR and apparently sporadic MTC. This locus could account for one-third to one-half of apparently sporadic pheochromocytomas and could modulate age of onset.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Results of the CAPP1 Study: aspirin and resistant starch are beneficial in familial adenomatous polyposis. *J. Burn*[1,3], *P.D. Chapman*[1], *D.T. Bishop*[2], *R. Dixon*[1], *F. Turner*[2], *J. Coaker*[1], *M.G. Dunlop*[4], *D. Eccles*[3], *A. Ellis*[3], *P. Fidalgo*[4], *H.F.A. Vasen*[5], *R. Fodde*[6], *G. Moslein*[4], *S. Bulow*[7], *R.K.S. Phillips*[8], *L. Bertario*[4], *G. Evans*[3], *J. Mathers*[1]. 1) School of Clinical Medical Sciences, University of Newcastle, Newcastle on Tyne, UK; 2) Cancer Research UK Genetic Epidemiology Unit, Leeds; 3) Cancer Research UK, Clinical Cancer Genetics Network, UK; 4) CAPP Consortium, c/o CAPP Office, University of Newcastle, Newcastle on Tyne, UK; 5) Foundation for Detection of Hereditary Tumours, Leiden, Netherlands; 6) Dept of Human & Clinical Genetics, Leiden University Medical Centre, Leiden, Netherlands; 7) Dept of Surgical Gastroenterology, Hvidovre Hospital, Copenhagen, Denmark; 8) Polyposis Registry, St Mark's Hospital, Harrow, UK.

In 1993 our consortium launched CAPP1, a European Concerted Action on Polyp Prevention. Carriers of Familial Adenomatous Polyposis (FAP) were recruited to a randomised placebo controlled trial of 600mg aspirin and/or 30 grams of resistant starch. Substantial epidemiological evidence supports the view that these interventions might prevent the development of adenomatous polyps, the precursor of colorectal cancer. 206 FAP carriers received treatment. Completed data on 133 subjects followed for at least one year have been analysed. Neither intervention resulted in a significant reduction in polyp number as assessed either by the endoscopist or blinded review of rectal videos. The mean size of largest polyps was, however, significantly reduced in the aspirin only group ($p=0.01$). A secondary analysis used data from those who had stayed more than one year, suggesting higher compliance. Here, both aspirin alone and the combined aspirin/resistant starch group achieved significance ($p=0.04$ and $p=0.03$).Those treated with starch had significantly shorter crypts ($p<0.0001$,95% CI 0.87,0.96); those treated with aspirin had longer crypts and a 37% increase in crypt cell proliferation. These data suggest that aspirin and resistant starch are protective against cancer but with different modes of action. Aspirin may act later, preventing progression of small adenomata.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 205 from 2003 ASHG Annual Meeting

Trisomy and the size of the oocyte pool: No connection? *J. Kline*[1,2], *A. Kinney*[1], *M.L. Reuss*[3], *A. Kelly*[2], *B. Levin*[2], *M. Ferin*[2], *D. Warburton*[2]. 1) NY State Psychiatric Institute, New York, NY; 2) Columbia University, New York, NY; 3) Bellevue Woman's Hospital, Niskayuna, NY.

The biologic basis for the increasing risk of trisomic conception with advancing maternal age is unknown. A prevalent hypothesis suggests that the age-related decline in the size of the oocyte pool leads to increased trisomy risk. We tested this hypothesis using three indicators of ovarian age that (a) reflect the size of the oocyte pool and (b) vary among women of the same chronologic age: the number of antral follicles, the level of dimeric inhibin B and the level of FSH. Women were ascertained through a spontaneous abortion or a livebirth at a hospital in upstate New York. The analytic sample consisted of 54 women with trisomic losses, 24 women with other chromosomally abnormal losses, 21 women with chromosomally normal losses, and 65 women with livebirths who were age-matched to the trisomy cases. Ovarian age indicators were usually measured after the second or third menstrual cycle following loss or our initial contact (for livebirths). We counted antral follicles using computerized images obtained from transvaginal ultrasound. Statistical analysis used age-stratified multiple linear regression models. All measures were associated with chronologic age as expected and showed considerable variability among women of the same age. However, neither antral follicle number nor levels of inhibin B and FSH differed between women with trisomic spontaneous abortions and any of the three comparison groups. This result does not support the hypothesis that oocyte pool size or associated hormonal changes are important determinants of trisomy risk.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Delineation of genomic breakpoints in chromosomal rearrangements: Lack of involvement of duplicated segments. *S. Schwartz[1], C. Astbury[1], J. Bailey[1], C. Crowe[2], E.E. Eichler[1], M. Eichenmiller[1], M. Graf[1]*. 1) Case Western Reserve Univ, and Univ Hosp of Cleveland,OH; 2) MetroHealth Med Ctr, Cleveland, OH.

With the progress made in the Human Genome Project over the last several years considerable knowledge has been gained about the structure of chromosomes. However, little is still known about the formation of most other abnormalities, such as terminal deletions and translocations. In order to better understand the mechanisms underlying these rearrangements, we undertook a study of 72 rearrangements (mostly terminal deletions and translocations), where we systematically mapped 110 breakpoints in these rearrangements using over 300 BACs and data in the UCSC Genome Browser. After using BACs to localize the breakpoints we wanted to determine if duplicated blocks of DNA were responsible for the formation of the rearrangements. To accomplish this, we compared breakpoints to: (1) the locations of DNA duplications predicted by three different in silico methods; (2) sites of potential duplication-mediated rearrangements; and (3) the location of genes as predicted by RefSeq. These results demonstrated that only 21 of the 110 breaks (19.1%) occurred in duplicated segments and 4.5% in predicted disease hot spots. Interestingly, 57 of the breaks (51.8%) appeared to occur within the genes defined by the RefSeq Gene track. The results from this study indicate a number of interesting findings including: (1) that the vast majority of chromosomal breaks (especially terminal deletions and translocations) are not mediated by blocks of duplicated DNA, consistent with our previous findings of breakpoints in six terminal deletions occurring within Line or Sine elements, but not duplicated DNA; (2) even when several deletions occurred at the same breakpoint, these did not involve the blocks of duplicated DNA; (3) the majority of tandem and inverted tandem chromosomal duplications studied did involve duplicated DNA; (4) more breaks appeared to occur within putative genes than originally expected. Even for cases of chromosomal deletions this is important as it might have an unexpected impact on the phenotype. It also may indicate that the underlying DNA structure in genes is more amenable to breakage.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 207 from 2003 ASHG Annual Meeting

Delineation of deletions and complexity in 'balanced' chromosome rearrangements: Occam's Razor bites the dust. *C. Astbury, E. Eichler, L. Christ, S. Schwartz.* Case Western Reserve Univ, Cleveland OH.

There is an approximate 7% risk of phenotypic abnormalities associated with balanced de novo rearrangements (Warburton 1991). Several hypotheses exist as to why these balanced rearrangements cause phenotypic abnormalities, such as a possible break in a gene or a cryptic deletion at the chromosomal breakpoints. High-resolution chromosome analysis in conjunction with FISH analysis of BACs has permitted the delineation of breakpoints in balanced chromosomal rearrangements. We report here 15 cases (2 prenatal, 13 postnatal) of 'balanced' rearrangements (10 translocations, 3 inversions, 2 insertions), ascertained with either abnormal ultrasounds or abnormal phenotypes. High-resolution chromosome analysis and FISH with BACs (over 350 in this study) were utilized to precisely define and delineate the rearrangements. Cryptic deletions (some as small as 3.5 Mb) were found in 9/15 cases, with the loss of 14 to 70 genes. In 5/15 cases, a known or putative gene was disrupted at one or more breakpoints, possibly leading to the phenotypic problem. In 1 case, neither deletion nor gene disruption could be detected. Also, 4 of these 15 cases, plus 2 other cases studied, demonstrated much more complexity than originally anticipated, with a minimum of 4 breaks within each rearrangement (one case had 8 different breakpoints), thus accounting for a much greater chance of gene loss or breakage. Our work has shown that based on high-resolution chromosome analysis, the available Human Genome data, and BAC FISH analysis, we have developed highly efficient and consistent methodology to study 'balanced' rearrangements. These data emphasize: (1) the importance of the Human Genome data to structural abnormalities; (2) the symbiosis of traditional cytogenetics with FISH analysis of BACs to precisely define abnormalities; (3) the importance of systematically analyzing all phenotypically abnormal cases with balanced rearrangements; (4) the presence of cryptic deletions as a cause of phenotypic abnormalities in such cases; and (5) a greater complexity to these rearrangements than initially anticipated, suggesting that the law of parsimony (Occam's Razor) does not hold in cytogenetics.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Chromosome 15 Proximal Pseudogene Cluster and Breakpoint Duplicons: Similar Phenomena at Different Evolutionary Time Points. *S.K. Mewborn[1], J.A. Fantes[2], D.H. Ledbetter[1,3].* 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) MRC, Human Genetics Unit, Edinburgh, UK; 3) Department of Human Genetics, Emory University, Atlanta, GA.

The proximal region of chromosome 15 contains many duplicated sequences. One class, the pseudogene cluster, are truncated copies of known genes located elsewhere in the genome and show polymorphic variation in copy number. Other sequences, the breakpoint duplicons, also consist of pseudogenes or gene fragments and are present in multiple copies throughout chromosome 15. These breakpoint duplicons are implicated in mediating the common deletions of 15q11-q13 resulting in Prader-Willi and Angelman syndromes and additional chromosomal rearrangements. ZooFISH analyses of clones from these regions on non-human primate species show that the proximal pseudogene cluster sequences arose later on chromosome 15 (8.2-23.3 Mya) than those associated with the deletions (30 Mya). Additionally, these proximal pseudogene cluster sequences did not all appear on chromosome 15 at the same point in evolutionary history. The Immunoglobulin H and the Neurofibromatosis I pseudogenes pre-date the BCL8A pseudogene. In contrast, different portions of the breakpoint duplicons reveal similar patterns in the ZooFISH analyses suggesting that these different pseudogenes or gene fragments may have arisen on chromosome 15 over a shorter time window than the proximal pseudogene cluster. The evolutionary histories of these two types of genomic duplicons on chromosome 15 show some similarities that may indicate a common mechanism in their transposition to chromosome 15. The pericentromeric region of chromosome 15 has continued to accumulate pseudogenes after the rapid expansion in copy number of the breakpoint duplicons. This accumulation of pseudogenes and gene fragments followed by expansion and dispersion may be an on-going genomic process.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Detection of deletions, duplications and triplications in two subjects with 1p36 chromosomal rearrangements by microarray-based comparative genomic hybridization. *L.G. Shaffer[1,2], B.C. Ballif[1], C.D. Kashork[1], H.A. Heilstedt[1], C.A. Shaw[1], W. Yu[1]*. 1) Dept Molecular & Human Genetics, Baylor Col Med, Houston, TX; 2) Health Res & Edu Ctr, Washington State Univ and Sacred Heart Med Ctr, Spokane.

Chromosome 1p duplications are rare, and to our knowledge, a chromosome 1p triplication has not been reported. There are 14 cases of 1p duplications reported in the literature, with most showing proximal interstitial duplications. We studied two subjects with apparent duplications of 1p36. Complex rearrangements of 1p36 were detected using microarray-based comparative genomic hybridization (array CGH). A 1p36 microarray was constructed from a contig of 97 large-insert BAC clones spanning 10.5 Mb of the most distal region of 1p36. The array also contained 41 clones from the subtelomeric regions of all chromosomes except the acrocentric short arms. In subject 1, array CGH identified a terminal triplication of ~1 Mb followed by a proximal duplication of ~4 Mb within 1p36. In subject 2, array CGH revealed a terminal deletion of ~200 kb followed by a triplication of ~2.6 Mb and a proximal duplication of ~450 kb. In addition, the 19q telomere was translocated to the most telomeric portion of the derivative chromosome 1. DNA copy number changes and breakpoint locations identified with array CGH were confirmed by metaphase and interphase FISH. These complex chromosomes may have formed premeiotically after double-strand breaks, sister chromatid fusions, and several breakage-fusion-bridge cycles. A final telomere capture event likely stabilized the rearrangements. The rearrangement in subject 1 was previously reported from our laboratory as a duplication based on a small number of FISH experiments. Thus, array CGH, comparable to 97 individual FISH experiments to 1p36, was necessary to uncover the complexity of this rearrangement. Our data demonstrate that array CGH is a powerful tool for characterizing unbalanced rearrangements through its ability to detect a variety of DNA copy number changes (del, dup, trp) and localize breakpoints.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Presence of interstitial or terminal telomeres at the rearranged sites of human constitutional chromosome anomalies. *F. Fortin*[1,3], *M. Beaulieu Bergeron*[1,3], *R. Fetni*[4], *N. Lemieux*[1,2,3]. 1) Département de pathologie et biologie cellulaire, Université de Montréal, Montréal, Québec, Canada; 2) Département de pathologie et; 3) Centre de recherche, Hôpital Sainte-Justine, Montréal, Québec, Canada; 4) Department of pathology, McGill University Health Center, Montreal, Quebec, Canada.

Chromosome healing is known to be a process allowing the rescue of chromosomes bearing a broken end and is thought to occur through telomere capture or de novo telomere addition. Telomere capture has been previously described in both constitutional and cancer chromosome deletions involving cryptic translocations. Interstitial telomeres have been reported in translocations and ring chromosomes, also in constitutional or in cancer samples. The goal of this study was to determine the frequency at which interstitial or terminal telomeres are observed in target constitutional chromosome anomalies. 37 cases were selected from 1991 to 2002 and were chosen according to the following criteria : terminal deletions, translocations or ring chromosomes, all involving the terminal bands of either short or long chromosome arms and a minimal resolution of 550 bands at karyotype analysis. 18 terminal deletion, 11 translocation and 8 ring chromosome cases were selected. FISH using a pan-telomeric probe recognizing the human telomere consensus sequence (TTAGGG)n, chromosome-specific subtelomeric probes and some locus specific probes were used. For healing of broken ends, 15/18 cases of terminal deletions showed the absence of the subtelomeres and the presence of telomeres. For interstitial telomeres, 2/11 translocation cases and 2/8 ring chromosome cases presented interstitial telomeres. Our study shows that interstitial telomeric sequences are uncommon in translocations and ring chromosomes, while the presence of terminal telomeres can be assessed in most of cases of terminal deletions and involves various chromosomes. It remains uncertain whether healing occurred through telomere capture or de novo telomere addition, but our study suggests that this phenomenon probably happens more frequently than previously thought.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 211 from 2003 ASHG Annual Meeting

L1 Retrotransposition associated with Telomere Repeats. *T.A. Morrish, J.V. Moran.* Dept Human Genetics, Univ Michigan, Ann Arbor, MI.

Retrotransposition competent L1s have a 5' untranslated region (UTR), two open reading frames (ORFs), and a 3'UTR that ends in a poly (A) tail. ORF1 encodes an RNA binding protein, while ORF2 encodes endonuclease (EN) and reverse transcriptase (RT) activities and contains a carboxyl-terminal cysteine-rich(C) domain of unknown function. L1s likely retrotranspose via target-site primed reverse transcription (TPRT). During this process, L1 EN nicks DNA at a consensus cleavage sequence (5'-TTTT/A) freeing a 3'OH that acts as a primer for reverse transcription of L1 RNA by the L1 RT. We recently described a pathway for L1 retrotransposition in Chinese Hamster Ovary (CHO) cells that depends on L1 RT, but is independent of L1 EN. EN-independent retrotransposition occurs at near wild-type levels in two mutant CHO cell lines that lack the non-homologous end joining (NHEJ) proteins XRCC4 and DNA-PKcs. When characterized from wild type and XRCC4-deficient cells, the EN-independent events had unusual structural features (i.e., frequent 3' truncations, a lack of target site duplications, integration into atypical target sequences, and occasional integration with cDNA fragments). Together, these data suggest that L1 can integrate into DNA lesions via an RNA or cDNA intermediate. Here, we characterized six EN-independent events from DNA-PKcs deficient cells. Besides being NHEJ-deficient, these cells have an increase in telomere-telomere fusions. Three events are structurally similar to those characterized from XRCC4-deficient cells, indicating they likely occur by a common mechanism. By comparison, the other three events contain telomere-repeat sequences. In two cases, 10 or 24 copies of a (5'-CCCTAA) repeat follow the L1 poly (A) tail. In a third case, a telomere-like sequence follows the L1 poly (A) tail. We also found that a mutant in the C-domain retrotransposes in both NHEJ deficient cell lines, and that some of the resultant events in DNA-PKcs deficient cells contain telomere-repeat sequences. Thus, our data suggest that L1 can retrotranspose into a telomere using an RNA or cDNA intermediate.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 212 from 2003 ASHG Annual Meeting

Development and validation of a pilot comparative genomic hybridization microarray for the application of clinical diagnosis. *W. Yu, C. Shaw, C. Kashork, M. Santini, C. Chinault, S. Cheung, A. Beaudet.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

DNA microarray-based comparative genomic hybridization (array CGH) is a powerful technology to identify chromosome aberrations throughout the entire genome. This is accomplished by co-hybridizing differentially labeled test and reference DNAs to a microarray of genomic clones. We have previously developed BAC DNA-based arrays for this purpose including a human 1p36 microarray spanning the most distal 10.5 Mb of chromosome 1 and human 17p arrays spanning the Charcot-Marie-Tooth disease type 1A and Smith-Magenis syndrome regions. These have been used to detect deletion, duplication and triplication in samples with previously well characterized chromosome copy number change by fluorescence in situ hybridization (FISH). Here we report the development and validation of a pilot array to explore the potential applications for clinical diagnoses. The array contains a total of 72 clones including 41 subtelomeric clones from all human chromosomes and 31 clones from genomic positions corresponding to 21 different genetic disorders. An optimized experiment protocol was established and array CGH was performed to examine 11 blood samples from patients with different genetic disorders in a blinded study. The array CGH data resulted in detection of all known chromosome aberrations revealed by FISH analysis as well as some additional chromosome imbalances that were not detected with the current standard practice. As expected, the main limitation of the array CGH was found to be its inability to detect chromosome changes such as balanced translocations or inversions. In summary, our data demonstrates that array CGH is an accurate, sensitive, fast approach to analyze many chromosome imbalances and it is feasible to use this approach for clinical diagnosis of genetic disorders.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 213 from 2003 ASHG Annual Meeting

Array comparative genomic hybridization: Applications in genetic medicine. *K.A. Rauen*[1,2], *M.E. Norton*[3], *R. Segraves*[1,4], *T.A. Tokuyasu*[1], *D. Pinkel*[1,4], *D.G. Albertson*[1,4], *P.D. Cotter*[2]. 1) Comprehensive Cancer Center, UC San Francisco, CA; 2) Pediatrics, UC San Francisco, CA; 3) Obstetrics and Gynecology, UC San Francisco, CA; 4) Laboratory Medicine, UC San Francisco, CA.

Array comparative genomic hybridization (CGH) provides a genome-wide scan for identifying DNA copy number variation. We report the evaluation of a high-resolution 2464 genomic microarray for identifying and characterizing cytogenetic abnormalities in prenatal and postnatal clinical cytogenetic samples. Postnatal samples included whole chromosome aneuploidy, chromosome deletions and duplications, unbalanced structural rearrangements and submicroscopic deletions. All chromosome aberrations were readily identified and showed copy number variation of all BACs on the appropriate chromosome. Deletions, duplications, and unbalanced structural rearrangements previously identified by molecular cytogenetics were confirmed by array CGH which additionally provided more detailed data at the molecular level, allowing more accurate sizing of these aneusomies. To determine the feasibility of this technique for routine prenatal diagnosis, we prospectively collected samples referred for cytogenetics analysis. DNA was isolated from uncultured amniocytes or chorionic villi. Array CGH was performed and results compared with conventional cytogenetic analysis. The samples included cytogenetically normal results, trisomies, a terminal deletion and a submicroscopic deletion. All cytogenetic abnormalities in this prenatal series were accurately detected with array CGH and in one case, more detail was provided at the molecular level. In addition to the detection of the cytogenetic abnormalities in these series, there were a few other clones in some of the cases that had a ratio indicating the possibility of an abnormality (not including clones that show frequent copy number polymorphism in the human population). These abnormal ratios may represent rare copy number polymorphisms or false positive indications of abnormality. We are continuing the development of the analytical criteria to increase the confidence with which measured ratio changes accurately reflect copy number changes in the genome.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 214 from 2003 ASHG Annual Meeting

Increased sensitivity for the detection of unbalanced submicroscopic rearrangements by array CGH (aCGH) compared to telomere FISH. *A. Wong[1], C. Lese Martin[2], S. Uhrig[2], K. Heretis[1], H.E. McDermid[3], T. Ruffalo[4], K. Wilber[4], W. King[4], D.H. Ledbetter[1].* 1) Dept Human Genetics, Emory Univ, Atlanta, GA; 2) Dept Human Genetics, Univ of Chicago, Chicago, IL; 3) Dept Biol Sci, Univ of Alberta, Edmonton, Alberta, Canada; 4) Abbott Vysis, Inc. Downers Grove, IL.

Genome-wide telomere screening using FISH has revealed that a significant proportion (~5%) of unexplained mental retardation is due to submicroscopic telomere imbalance. However, telomere FISH is labor intensive and can be difficult to incorporate with other routine laboratory procedures. We evaluated aCGH as an alternative approach that might be more cost-effective if reliable and sensitive. We selected genomic BAC/PAC clones that mark the most distal, unique regions of each chromosome end, as well as clones for a molecular ruler strategy to delineate the size of genomic imbalances. We designed a custom telomere array containing all telomere clones as well as molecular ruler clone sets from 1p, 16p, 17p, and 22q telomeres. We labeled patient DNA with Cy-3 and co-hybridized with normal reference DNA labeled with Cy-5 on both the telomere array and the Vysis GenoSensor (GSA) 300 array. We previously performed telomere FISH on over 500 patients with unexplained mental retardation and a normal G-banded karytoype. From this patient group, a blinded study was designed including 23 abnormal and 70 normal telomere FISH cases for aCGH analysis. The aCGH accurately identified all 23 abnormal cases, including 12 terminal deletions, 4 unbalanced translocations, and 4 cases of partial trisomy 16p. Surprisingly, the aCGH study also identified 4 duplications that were *not* detected by FISH analysis. These included duplications on 4q and 10q telomeres, and one case with duplication of a clone from the cat-eye syndrome region on chromsome 22 (GSA 300 array). These results show that aCGH and telomere FISH performed equally well for deletion detection, but the aCGH outperformed telomere FISH in identifying subtle duplications. Since aCGH is potentially more amenable to automation than genome wide FISH approaches, it may provide a more cost-effective and sensitive approach to a molecular karyotype.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Mutations in *ENPP1* cause idiopathic infantile arterial calcification. *P. Nurnberg*[1], *F. Rutsch*[2], *S. Vaingankar*[3], *N. Ruf*[1], *R. Terkeltaub*[3]. 1) Gene Mapping Center, Max Delbrueck Center for Molecular Medicine, Berlin-Buch, Germany; 2) University Children's Hospital, Muenster, Germany; 3) Department of Medicine, Veterans Affairs Medical Center, UCSD, La Jolla, CA.

Idiopathic infantile arterial calcification (IIAC) is characterized by calcification of the internal elastic lamina of large and medium-sized muscular arteries and stenosis due to myointimal proliferation. Sometimes peri-articular calcification is also observed. Although survival to adulthood has been reported, most patients die within the first six months of life. Several cases of affected siblings have been reported suggesting a recessive genetic defect (OMIM 208000). Tiptoe-walking *ttw/ttw* mice also develop articular cartilage, synovial, perispinal ligament, and aortic calcifications in early life. Recently, a nonsense mutation in the *Enpp1* gene was identified as cause of the *ttw* phenotype. *Enpp1* encodes ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1). This cell surface enzyme generates inorganic pyrophosphate (PP_i), a solute that regulates cell differentiation and serves as an essential physiologic inhibitor of calcification. Here we demonstrate that IIAC results from recessive mutations inactivating ENPP1 in humans. We analyzed patients from 11 unrelated IIAC kindreds and identified four different mutations resulting in premature termination codons (PTCs) and nine additional missense mutations disseminated over the coding region. If different mutations were identified in a patient, trans status was proven either by analysis of the parents DNA or by determination of allele-specific transcript levels using QUASEP (**QU**antification of **A**llele-**S**pecific **E**xpression by **P**yrosequencing). Structural data and functional analyses suggest that all PTC-causing and eight of the nine missense mutations are pathogenic. Our results point to disturbed PP_i regulation as a major factor in arterial calcification.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Mutations in Capillary morphogenesis protein 2 cause hyaline deposition disorders. *N. Rahman[1], S. Hanks[1], S. Adams[1], J. Douglas[1], A. Superti-Furga[2], PA. Futreal[3], Hyaline Deposition Disorders Collaboration.* 1) Section of Cancer Genetics, Institute of Cancer Research, London, United Kingdom; 2) Division of Molecular Pediatrics, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; 3) Cancer Genome Project, The Wellcome Trust Sanger Institute, Hinxton, Cambs, UK.

Juvenile hyaline fibromatosis (JHF) and infantile systemic hyalinosis (ISH) are autosomal recessive conditions characterised by multiple subcutaneous skin nodules, gingival hypertrophy, joint contractures and hyaline deposition. We used homozygosity mapping to localise the gene for JHF and ISH to chromosome 4q21, and identified 15 different deleterious mutations in Capillary Morphogenesis protein 2 (*CMG2*) in 17 families. CMG2 is a transmembrane protein that is induced during capillary morphogenesis and that binds laminin and collagen IV via a von Willebrand A (vWA) domain. Of interest, CMG2 also functions as a cellular receptor for anthrax toxin as does the related protein TEM8, which was initially identified as a gene differentially upregulated in malignancy. We have identified a novel, highly conserved cytoplasmic domain that is the defining sequence hallmark of this protein family. Furthermore, genotype-phenotype analyses suggest that abrogation of binding by the vWA domain results in severe disease typical of ISH, whereas in-frame and missense mutations affecting the novel cytoplasmic domain result in the milder JHF phenotype. These data demonstrate that juvenile hyaline fibromatosis and infantile systemic hyalinosis are allelic conditions and implicate perturbation of basement membrane matrix assembly as the cause of the characteristic perivascular hyaline deposition seen in these conditions. Email: nazneen@icr.ac.uk.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

The classic form of congenital fibrosis of the extraocular muscles (CFEOM1) is caused by heterozygous mutations in *KIF21A*. *K. Yamada, M.P. Rogines-Velo-Sardi, C. Andrews, W-M. Chan, E.C. Engle.* Children's Hospital and Harvard Medical School, Boston, MA.

Congenital fibrosis of the extraocular muscles, CFEOM1, is an autosomal dominant syndrome characterized by congenital bilateral infraducted ophthalmoplegia with ptosis. Our previous studies mapped the CFEOM1 disease gene (*FEOM1*) to12cen and identified the pathology as absence of the superior division of the oculomotor nerve and the corresponding alpha-motoneurons in the midbrain, with marked changes in the levator palpebrae superioris and superior rectus. Affected individuals from 18 ethnically diverse *FEOM1*-linked CFEOM1 pedigrees were screened for mutations. After eliminating three candidate genes, we identified and determined the structure of a fourth positional candidate gene, *KIF21A* the human ortholog of mouse *Kif21a* and a member of the kinesin superfamily. The classic kinesins (including KIF21A) have a motor domain that moves the kinesin and its cargo along the microtubule, a stalk region, and a tail region where the kinesin interacts with its cargo. Mouse Kif21a is known as a neuronally enriched kinesin involved in axonal transport. Sequence analysis identified *KIF21A* mutations in all 18 pedigrees. These mutations co-segregated with the disease and were not present in 105 unrelated controls. Sixteen pedigrees harbored mutations at one of two nucleotide positions in the same amino acid, and two pedigrees harbored unique mutations. The pedigrees with the same mutations did not share a common disease-associated haplotype and, in two, we established that the mutations were *de novo*, suggesting that these mutations arose independently and that these amino acids are mutational hot spots. Three of the four mutations, including the recurrent mutations, are located in a specific region of the KIF21A stalk and suggest a functional importance for this region. We have raised an antibody to human and mouse KIF21A and determined that the protein is expressed in both the oculomotor nucleus and extraocular muscle. We are now studying affected tissues to determine how these mutations result in CFEOM1. This is the first report of kinesin mutations causing a developmental disorder.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Mutations in the HFE2A gene cause juvenile hemochromatosis. *P. Goldberg[1,2], G. Papanikolaou[3], E. Ludwig[1], M. MacDonald[1], L. Andres[1], M.-P. Dube[1], P. Franchini[1], R. Babakaiff[1], J. Risler[1], C. Zaborowska[1], S. Pimstone[1], P. Brissot[4], G. Lockitch[5], M. Hayden[1,2], M. Samuels[1]*. 1) Xenon Genetics, Burnaby, BC, Canada; 2) Dept of Medical Genetics, Univ of British Columbia, Vancouver, BC, Canada; 3) First Dept of Medicine, Univ of Athens, Athens, Greece; 4) Univ Hospital, Pontchaillou, Rennes, France; 5) Department of Pathology & Laboratory Medicine,Children's & Women's Health Centre of British Columbia, Vancouver, BC, Canada.

Juvenile hemochromatosis (JH, OMIM 602390) is an autosomal recessive disorder characterized by early onset iron overload, cardiomyopathy and hypogonadism. A locus for JH has been genetically mapped in several populations, to a region near the centromere of chromosome 1q. The human genome assembly in this region is problematic, with multiple unlinked contigs, extensive local duplication, and uncertain gene content. We refined the mapping of the 1q JH locus in Greek families using a set of novel microsatellite markers, achieving a sumlod of 4.05 and a minimal interval of approximately 1.7 MBp (contingent on the exact structure assumed for the assembly). Sequencing identified multiple mutations in one gene (HFE2A) mapping within the interval. Subsequent analyses including Canadian and French families yielded a total of six mutations including four missense variants, one nonsense variant, and one frameshift. All affected individuals were either homozygotes or compound heterozygotes. There is no evidence of a phenotype in heterozygotes for any of the mutations. None of the mutations was observed in a set of 95 Greek plus 125 northern European normal controls. Since hepcidin mutations have been implicated in other JH patients, it is likely that the 1q JH gene may also be involved in the hepcidin pathway. Further work to define the role of HFE2A in iron metabolism and the hepcidin pathway is underway.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 219 from 2003 ASHG Annual Meeting

Hermansky-Pudlak syndrome type 7 (HPS-7) results from mutations in dysbindin, a new member of the biogenesis of lysosome-related organelles complex 1 (BLOC-1). *W. Li*[1], *Q. Zhang*[1], *N. Oiso*[2], *E.K. Novak*[1], *R. Gautum*[1], *E.P. O'Brien*[1], *C.L. Tinsley*[3], *D.J. Blake*[3], *R.A. Spritz*[2], *N.G. Copeland*[4], *N.A. Jenkins*[4], *D. Amato*[5], *B.A. Roe*[6], *M. Starcevic*[7], *E.C. Dell'Angelica*[7], *R.W. Elliott*[1], *V. Mishra*[8], *S.F. Kingsmore*[8], *R. Paylor*[9], *R.T. Swank*[1]. 1) Dept Mol & Cell Biol, Roswell Park Cancer Inst, Buffalo, NY; 2) Univ of Colorado Health Sciences Center, Denver, CO; 3) Univ of Oxford, Mansfield Road, Oxford, UK; 4) NCI, Frederick, MD; 5) Mount Sinai Hospital, Toronto, ON, Canada; 6) Univ of Oklahoma, Norman, OK; 7) UCLA School of Medicine, Los Angeles,CA; 8) Univ of Florida, Gainesville, FL; 9) Baylor College of Medicine, Houston, TX.

Hermansky-Pudlak Syndrome (HPS; MIM 203300) is a genetically heterogeneous disorder characterized by oculocutaneous albinism, prolonged bleeding and pulmonary fibrosis due to abnormal vesicle trafficking to lysosomes and related organelles such as melanosomes and platelet dense granules. In mice there are at least 16 HPS loci including sandy (*sdy*). Here we show that mutation of the dysbindin gene (*DTNBP1*) causes a novel form of HPS (HPS-7) in a Portuguese patient and that the sandy (*sdy*) mouse HPS mutant expresses no dysbindin protein due to a deletion within *Dtnbp1*. Dysbindin is a ubiquitously expressed protein that binds to - and -dystrobrevins, components of the dystrophin-associated protein complex (DPC) in both muscle and nonmuscle cells. Further, we show that dysbindin is a component of a protein complex, BLOC-1, which regulates trafficking to lysosome-related organelles and includes the mouse HPS proteins pallidin, muted and cappuccino. These findings provide the first evidence for the importance of BLOC-1 in the production of the HPS phenotype in humans, demonstrate a role for dysbindin in the biogenesis of lysosome-related organelles and reveal unexpected interactions between components of DPC and BLOC-1. We also have some evidences to show that a larger heterogeneous protein complex containing BLOC-1, we named it as Blosome, may exist in higher eukaryotic organisms to govern the biogenesis of lysosome-related organelles. It remains controversial that *DTNBP1* is the candidate gene of schizophrenia.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 220 from 2003 ASHG Annual Meeting

A novel family of extracellular matrix proteins implicated in Fraser syndrome and the mouse blebbing mutants.

P. Scambler[1]*, S. Jadeja*[1]*, L. McGregor*[1]*, S. Darling*[1]*, M. van Haelst*[1]*, G. Chalepakis*[2]*, S. Vrontou*[2]*, V. Makela*[3]*, R. Winter*[1]. 1) Molecular Medicine Unit, Inst Child Health, London, United Kingdom; 2) Institute of Molecular Biology and Biotechnology, FO.R.T.H., Heraklion 71110, Crete, Greece; 3) Karolinska Institute, Stockholm, Sweden.

Fraser syndrome (FS) commonly presents with cryptopthalmos, syndactyly and renal defects. We recently showed that FS was due to mutations in the *FRAS1* gene which encodes a putative extracellular matrix protein. The mouse blebbing mutants, of which 5 have been described, were proposed as a mouse model of FS and we discovered *Fras1* mutation in the *bl* strain. Here, we report the identification of 4 *FRAS1*-like genes. One, *Fras2*, is at the mylencephalic bleb (*my*) locus and is mutated in two FS pedigrees. Thus, FS, like blebbing, is genetically heterogeneous. TEM analysis reveals the same basement membrane defect in *mymy* and *blbl* embryos, with separation of the dermal cellular layer from the basement membrane in a manner reminiscent of dystrophic epidermolysis bullosa. Lack of Fras2 protein affects the rate of would healing in mutant embryos, but *my/bl*. Transheterozygotes animals are apparently normal. *mymy* homozygotes on the NMRI background are semilethal and some surviving homozygotes appear normal. Fras2 lacks the N-terminal VWFC and furin domains of Fras1, which may indicate a more restricted range of function. Another family member, *Fras3*, is linked to head blebs (*heb*), the lack of any transmembrane domain suggesting this gene encodes a secreted protein. Further analysis of this gene family will enhance our understanding of epithelial integrity and renal development, with potential implications for wound healing. The effect of compound mutation at *Fras* loci is currently being analysed.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Identification of neuroD as a downstream effector of abnormal lung morphogenesis in a mouse model of Marfan syndrome. *E.R. Neptune*[1], *A.C. Russell*[1], *J.H. Cho*[2], *M.J. Tsai*[2], *H.C. Dietz*[1, 3]. 1) Inst Genetic Med, Johns Hopkins Univ SOM, Baltimore, MD; 2) Dept Molec & Cell Biol, Baylor COM, Houston, TX; 3) HHMI.

A subgroup of patients with Marfan Syndrome (MFS) have recurrent pneumothoraces and progressive emphysematous changes. We recently reported that fibrillin-1 deficient mice, a murine model of MFS, display primary impairment of alveolar septation that correlates with enhanced TGF activation and signaling and is rescued by TGF antagonism in vivo. In order to identify downstream effectors of septation impairment, we applied a rigorous expression profiling strategy using lungs from homozygous-targeted fibrillin-1 deficient pups and their wild-type littermates. First, we identified expression changes that attend normal septation between postnatal days 1 and 5 in wild-type animals. We then screened for discordant responses in fibrillin-1 deficient animals, identifying more than 150 transcripts that displayed perturbed expression profiles in mutant mice. Transcripts that were downregulated in mutant mice but upregulated in control animals at day 5 were stratified as permissive for septation. Conversely, transcripts that were upregulated in mutant lungs but downregulated in control animals were coded as inhibitory for septation. Particular significance is given to permissive molecules with restored expression in mutant animals treated with TGF-neutralizing antibody. One permissive transcript identified is neuroD, a bHLH transcription factor expressed in neuroendocrine cells. Notably, pulmonary neuroendocrine cells secrete neuroactive peptides, such as bombesin, that are thought to be involved in normal lung maturation. By immunoblotting and northern analysis, we confirmed reduced expression of NeuroD in the fibrillin-1 deficient lung at relevant developmental stages. Histologic and morphometric analysis of NeuroD-targeted mice demonstrated perinatal airspace enlargement consistent with impairment of distal lung septation. Our studies demonstrate the utility of expression profiling analysis of organ-specific disease phenotypes to identify novel mediators of tissue morphogenesis. Here, we have implicated neuroD in both normal distal lung development and the pathogenesis of MFS.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 222 from 2003 ASHG Annual Meeting

Perturbation of multiple 1 integrin-mediated signaling cascades in the pathogenesis of cerebral vascular malformations.
J. Zhang[1], R.E. Clatterbuck[1], B. Sharmila[1], D. Rigamonti[1], H.C. Dietz[2]. 1) Neurosurgery, John Hopkins University, Baltimore, MD; 2) JHMI and Inst of Genetic Med, Johns Hopkins SOM, Baltimore, MD.

Cerebral cavernous malformation (CCM) is a dominant microvascular disorder characterized by venous sinusoids that predispose to intracranial hemorrhage. Mutations in the gene KRIT1 account for the majority of familial CCM cases; however, little is known about the cellular functions of Krit1. Krit1 (Krev Interaction Trapped 1) was originally identified through its interaction with the Ras-family GTPase krev1, inferring a role in GTPase signaling cascades. We demonstrated that full-length krit1does not interact with krev1, but rather interacts with icap1, a modulator of 1 integrin signal transduction. This suggests a role for krit1 in 1 integrin-mediated angiogenesis. Using RNA interference (RNAi), we have specifically silenced krit1 expression in cultured human endothelial cells and observed dramatic perturbations in cellular proliferation, mobility, and survival. Each of these abnormalities was specifically correlated with alteration in the phosphorylation status and inferred activity of specific kinases and substrates that comprise specific 1 integrin-modulated cellular signaling networks. For example, a defect in the MAP kinase cascade in krit1-depleted cells manifests with decreased phosphorylation of RAF, MEK and ERK, known effectors of integrin-mediated cellular proliferation. Concordant but less severe abnormalities were seen in icap1-depleted cells, suggesting that krit1 and icap1 both positively regulate these events, rather than providing counter-regulation (as previously inferred). Using co-immunoprecipatation (co-IP), we also found a new krit1-interacting partner that contributes to the signaling cascade that mediates enhanced apoptosis in krit1-dpeleted cells. Finally, we show that full-length krit1 colocalizes with icap1 in both the nucleus and the cytoplasm. This mechanistic understanding of abnormal cellular performance in cerebral vascular malformation will likely facilitate identification of other CCM genes and the exploration of novel and rational therapeutic strategies.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 223 from 2003 ASHG Annual Meeting

Recurrence risk and asymptomatic and symptomatic mosaicism in Ehlers Danlos syndrome (EDS) type IV. *P.H. Byers, M.G. Pepin, U. Schwarze, L.K. Gaulke.* University of Washington, Seattle, WA.

EDS type IV is a dominantly inherited disorder complicated by vascular and bowel rupture and shortened life expectancy that results from mutations in the COL3A1 gene, which encodes the chains of type III procollagen. We have identified the causative COL3A1 mutation in 275 of the 334 probands we have studied. About 40 % of the individuals identified represent the first known affected person in their families. We identified 135 families for which we had sufficient information to calculate recurrence risk after birth of a proband with two unaffected parents. In those families there were 198 siblings, of whom 14 were affected in 12 families; in 10 families there was a single recurrence and in 2 families there were 2 additional affected children for a measured recurrence rate of 7.1 %. In 4 of the 12 families with recurrence we confirmed that one parent was mosaic in blood in 3 (all mothers) and in one other identified the source of the mutant allele as the mother, who was not measurably mosaic in white cell DNA (5% of the mutant allele). In 23 families in which there had not been recurrence among siblings, we identified 5 (22%) in which one parent (3 mothers and 2 fathers) was mosaic for the mutation, consistent with the measured recurrence risk. In mosaic individuals, the proportion of the affected allele in white blood cell DNA ranged from 30% to undetectable. None of the mosaic parents, aged from 30 to 51 at the time of their study, was symptomatic. We identified one symptomatic (bowel rupture) 17-year-old male who was mosaic for a mutation; 30% of alleles in fibroblasts and white blood cells. In EDS type IV tissues are affected in a localized fashion so that a population of cells with a mutant allele in vessels or bowel wall could, like localized malignancy, be sufficient to become symptomatic but may require a broad patch of cells rather than the clonal expansion seen with malignant transformation. The recurrence rate after birth of an affected child is not always estimated by the level of mosaicism in parental blood because of isolated germline mosaicism. The risk for complications of EDS type IV to mosaic parents remains unknown.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 224 from 2003 ASHG Annual Meeting

Molecular diagnosis of Treacher Collins Syndrome by DNA sequencing reveals a spectrum of *TCOF1* mutations and non-penetrance. *S.H. Katsanis[1], B. Karczeski[1], D.M. McDonald-McGinn[2], D. Driscoll[2], I. Krantz[2], A. Kellogg[1], S. Audlin[1], C. Boehm[1], X. Wang[1], E.W. Jabs[1], E. Zackai[2], G.R. Cutting[1].* 1) Inst Genet Med, Johns Hopkins Univ, Baltimore, MD; 2) Children's Hosp of Phil, Univ of Penn Sch of Med, Philadelphia, PA.

Treacher Collins Syndrome (TCS) is an autosomal dominant craniofacial disorder characterized by downward slanting of the eyes, eyelid coloboma, absence of the lower eyelashes, hypoplastic zygomas, microtia, conductive hearing loss, and micrognathia. Molecular testing of TCS has been complicated by the high rate of *de novo* mutations in *TCOF1* and the absence of a mutation hotspot, except a single 5bp deletion in 16% (27/163) of reported cases. We have validated an assay to screen the entire coding sequence of *TCOF1*. Here we report the implementation of this protocol to test 25 patients submitted to our laboratory with indications for a clinical diagnosis of TCS. We have found *TCOF1* mutations in 20/25 of the patients analyzed (80%); 15 are novel (71%) comprising of 5 splice site, 5 frameshift, 3 missense, and 2 nonsense mutations. We found no evidence of a pathogenic mutation in *TCOF1* in the remaining 5 patients, with 4 having no family history or confirmed diagnosis of TCS. In the remaining family, although 4 family members exhibit some symptoms of TCS, other findings are not consistent with TCS and a diagnosis remains uncertain. Interestingly, in one family with 5 affected members, we found the only reported recurrent mutation in *TCOF1* (4138delAAGAA) in a mother and son with obvious TCS feautures. Prenatal diagnosis for this family predicted an affected child with the *TCOF1* mutation; however, at birth the child did not exhibit any manifestations of TCS. Retesting confirmed the molecular diagnosis. Although variable expressivity has been documented for *TCOF1* mutations, to our knowledge this is the first report of non-penetrance documented by molecular analysis. Given the spectrum of mutations across *TCOF1*, our findings demonstrate that direct sequencing is the most sensitive approach to confirm the molecular basis of TCS, but that variable expressivity and non-penetrance must be considered in the prenatal setting.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 225 from 2003 ASHG Annual Meeting

Chromosomal co-localization of genes with similar expression in mammalian evolution. *C.M. Malcom*[1], *G.J. Wyckoff*[2]. 1) Dept. of Anthropology, U. of Chicago, Chicago, IL. 60637; 2) Mol. Biol. Biochem., U. Missouri Kansas City, Kansas City, MO 64110.

In mammals, the level of functional constraint on genes is known to correlate with their breadth of expression (Duret & Mouchiroud, 2000). Additionally, levels of functional constraint are shared among positionally linked genes (Malcom et al., 2003). We examine the relationship between gene location (on chromosomes and within synteny bins), gene expression pattern, and substitution rate. A set of 3,921 orthologous genes between humans and mice that had well-curated synteny bin information was obtained. The coding region of all orthologs was aligned in-frame and the rates of synonymous and nonsynonymous change per nucleotide (Ks and Ka, respectively) and the level of constraint (Ka/Ks) were calculated (Li 1993). Gene expression information was gathered from Unigene and reannotated. Each gene was classified according to 39 descriptors-an expressed/not expressed indicator for 38 tissues and a variable for the number of tissues in which each gene is expressed. A Discriminant function analysis showed that these descriptors correctly predicted the human chromosome on which genes were located 10.2% of the time, twice the proper assignment by chance. When gene location in synteny bins (193 bins between human and mouse) was predicted, 9.3% of genes were correctly localized; 20 times better than by chance. We collected additional expression information from the Stanford Microarray database and, combining this information with Ks as an additional predictor, genes were correctly assigned to synteny bins 15.2% of the time; 30 times better than random. These results suggest that gene characteristics are likely to be shared across evolutionarily conserved regions, rather than whole chromosomes. Genes that are positionally linked may be coordinately expressed over long periods of evolutionary time. We hypothesize that modifiers of gene expression might be preserved over mammalian evolution, or synteny bins in which genes have similar expression are selectively preserved. These findings are relevant to further expression analysis, model organism studies, and disease gene mapping.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 226 from 2003 ASHG Annual Meeting

Gene expression from the aneuploid chromosome in a segmental trisomy mouse model of Down syndrome. *R. Lyle, C. Gehrig, S. Deutsch, S.E. Antonarakis.* Dept Medical Genetics, Centre Medicale Univ, Geneva, Switzerland.

Human autosomal aneuploides are very common but only Ts21 (Down syndrome, DS) normally survives beyond the early postnatal period. Since the genes on the triplicated chromosome 21 (Hsa21) are normal, DS is considered as a gene-dosage problem.There are two main hypotheses of how trisomy 21 leads to DS. First, individual genes which are triplicated are overexpressed leading directly to aspects of the DS phenoype. Alternatively, the increase in expression of a large number of genes from Hsa21 results in changes in global gene expression depending on environmental factors and genetic background.Our aim in this study was to accurately measure the expression of a large number of genes from the aneuploid chromosome to determine the consequences of trisomy at the level of gene expression.We have used Taqman real-time PCR to study the expression of the genes triplicated in the Ts65Dn mouse model of DS. Ts65Dn mouse is trisomic for approximately 14 Mb of mouse chromosome 16 and replicates many aspects of the phenotype which characterise DS. We studied a total of 99 genes in two developmental stages (P30 and 11 month) and six different tissues (brain, heart, kidney, liver, lung and muscle).For each tissue and stage the mean relative expression (Ts/Eu) is approximately 1.5. However, 63% of the genes are expressed at less than the theoretical value of 1.5, suggesting that the majority of genes are subject to incomplete dosage compensation. 18% of genes are expressed at a level greater than 1.5. There is significantly greater variability of expression in Ts compared to Eu individuals suggesting a general disruption of gene expression patterns. In contrast, there is significant correlation in gene expression between many tissues. This indicates that the altered expression levels are under the control of specific genetic programs for each gene. That is, the changes in expression are not stochastic, but are very similar for most genes across most tissues.This is the first systematic study at gene expression from the aneuploid chromosome in trisomy and is an important step in understanding how trisomy for Hsa21 leads to DS.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Computational analysis of the total transcription factor gene content of the human genome and the design of a microarray to interrogate their expression. *D.N. Messina, J. Glasscock, W. Gish, M. Lovett.* Department of Genetics, Washington University, St. Louis, MO.

Transcription factors (TFs) play a critical role in gene regulation and many play an important role in human genetic diseases. However, to date, no single, reasonably comprehensive database of human transcription factor genes exists. Here we describe an approach for identifying a comprehensive set of human transcription factor genes and the use of this information to prepare custom oligonucleotide microarrays so that their gene expression can be assayed in a single hybridization experiment. We gathered known transcription factor genes from three separate databases: TRANSFAC, LocusLink, and FlyBase. After removal of redundancies we used a seed set of 1476 sequences to search (by WU-BLAST) for human orthologs in the SCRIPTSURE database of Expressed Sequence Tags (ESTs). This EST database consists of all human ESTs nucleated to the human genome DNA sequence and it thus removes much of the redundancy and ambiguities associated with other EST sets. Positive hits were additionally checked by conceptual translation and comparison with DNA-binding domains extracted from the Pfam protein motif database. Three criteria were used to identify TFs: 1) target ESTs must be highly similar to an existing TF gene sequence; 2) multiple spliced forms must underly the EST cluster; and 3) the EST cluster must contain a DNA-binding domain. In addition to orthologs of the original seed set we also detected ~500 paralogs, resulting in a total of 1979 human transcription factor genes. Amongst these, by far the largest family consists of zinc finger TFs (773 members), followed by homeobox (199 members) and basic helix-loop-helix genes (118 members). From the set of 1979 genes we designed a microarray of 50mer oligonucleotide probes targeted to unique regions within the protein coding sequences of every TF. We have successfully used this array to interrogate TF gene expression across species as distant as chicken, mouse and man, as well as investigating several human disease states.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Heritability of gene expression in humans: A study of lymphoblastoid cell lines from twins. *J. Li*[1], *N. Risch*[2], *R.M. Myers*[1,2]. 1) Stanford Human Genome Ctr, Palo Alto, CA; 2) Department of Genetics, Stanford University School of Medicine,Palo Alto, CA.

Phenotypic variation is shaped by both genetic and non-genetic factors. In analyzing the genetic basis of any trait, one of the initial tasks is to assess its heritability, which measures how much of the observed variation in the population can be explained by inherited factors. While the heritability of many human traits and diseases has been estimated, the extent of genetic influence of gene expression is still not known. To address this question, we used cDNA microarrays and real-time RT-PCR to compare transcript levels in lymphoblastoid cell lines of 24 human twin pairs, including 14 monozygotic (MZ) and 10 dizygotic (DZ) pairs. For ~20,000 cDNAs that met our quality criteria on cDNA microarrays, the average intraclass correlation is 0.16 among the MZ twins, and 0.07 among the DZ twins. The heritability estimate, averaged over all genes, is 0.18, and is significantly above zero. The distribution of the heritability values among all genes showed a moderate and homogeneous positive shift that affects the majority of genes, with no evidence for a significant sub-population of genes having distinctively larger heritability values. We have also found no trend that genes with large inter-individual variations tend to have greater correlation values among twins. However, we did find that genes that are near each other in physical location tend to have positively correlated expression patterns. Analysis of gene function by using Gene Ontology annotations revealed that "transcription regulators" had moderately lower heritability values than average, suggesting greater gene-environmental interaction for these genes. Quantitative RT-PCR results on ~100 genes also showed greater correlation among MZ twins than among DZ twins, and yielded heritability estimates that were similar to the microarray results. Taken together, our results provide strong evidence that there is an extensive genetic influence of gene expression in humans, indicating that transcript level is a quantitative phenotype suitable for genome-wide genetic analysis.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 229 from 2003 ASHG Annual Meeting

GENETICS OF QUANTITATIVE VARIATION IN HUMAN GENE EXPRESSION. *V.G. Cheung[1,2], T.M. Weber[1], K.Y. Jen[1], M. Morley[1], J.L. Devlin[2], K.G. Ewens[2], R.S. Spielman[2].* 1) Dept of Pediatrics, Univ of Pennsylvania/Children's Hosp of Philadelphia, Philadelphia, PA; 2) Dept of Genetics, Univ of Pennsylvania, Philadelphia, PA.

The aims of this project are 1) to identify the genes whose expression levels are most variable among normal individuals, 2) to determine how much of the variation in expression phenotypes can be attributed to inherited (germ line) genetic differences among individuals and 3) to identify the determinants of expression variation.We first measured the expression of about 8,000 genes in lymphoblastoid cells (LCLs) from 150 individuals (50 parents and a replication set with 100 grandparents from CEPH families) using microarrays. Among the genes expressed in LCLs, we selected for analysis the genes that vary the most among individuals, since we expect highly variable phenotypes to be most amenable to genetic dissection. Among the highly variable genes are some that are expected, such as those in the HLA region and members of the P450 superfamily, that are known to be variable at the DNA level. For several of the highly variable genes, we found evidence of familial aggregation. We found the largest variance among unrelated individuals and the smallest variance between monozygotic twins, strongly suggesting that there is a genetic component to the variation in gene expression. We also identified genes whose expression levels are highly correlated between parents and their children.To identify the determinants of gene expression variation, we used a two-pronged approach that integrates genetic and computational methods. Hierarchical clustering shows that there are groups of highly correlated genes suggesting that they are co-regulated. We performed both association and genome-wide linkage analyses to map the expression control elements for several expression phenotypes. In most cases, data suggest trans-acting control elements rather than cis-acting controls. Our findings demonstrate that expression phenotypes are excellent models for genetic analysis of complex traits.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 230 from 2003 ASHG Annual Meeting

WARNING: siRNAs can induce off-target effects in mammalian cells. *P.C. Scacheri[1], O. Rozenblatt-Rosen[2], N. Caplen[1], T. Wolfsberg[1], M. Meyerson[2], F.S. Collins[1].* 1) National Human Genome Research Institute, NIH, Bethesda, MD; 2) Department of Medical Oncology, Dana-Farber Cancer Institute, and Department of Pathology, Harvard Medical School, Boston, Massachusetts.

The discovery that double-stranded short interfering RNA (siRNA) molecules can silence targeted genes has led to rapid adoption of this technology for analysis of gene function. The gene for multiple endocrine neoplasia, type 1 (MEN1) encodes a tumor suppressor protein of unknown function called menin. To determine the physiological role of menin, we designed siRNAs targeted to the MEN1 gene and tested their ability to silence menin in HeLa cells. Microarray analyses of RNA from menin-silenced cells suggested that the levels of p53 and p21 were altered in response to reduced menin levels. To follow-up the observed changes in p53/p21 expression, we tested 9 additional siRNAs targeted to different regions of the MEN1 gene. Unexpectedly, we detected dramatic and divergent changes in the protein levels of p53/p21 that were unrelated to menin silencing. Of the four siRNAs that consistently reduced menin levels, one upregulated p53/p21, one downregulated p53/p21, and two showed no change relative to mock transfected. Six siRNAs that did not reduce menin showed similar variations in p53/p21 levels. Dramatic changes in p53/p21 expression were also observed upon transfecting the MEN1-siRNA duplexes into MCF7, CaSki, and SiHa cells, suggesting that the effects were not limited to HeLa cells. These data indicate that siRNAs can induce non-specific effects that are sequence dependent. As p53 and p21 are activated in response to a variety of cellular stresses, it is difficult to identify the mechanism underlying this phenomenon. Possible explanations include the activation of interferon and RNA-dependent protein kinase (PKR) antiviral pathways, partial complementary matches to off-target genes, and siRNAs functioning as miRNAs in post-translational silencing. These results underscore the importance of utilizing more than one siRNA in functional analyses of mammalian genes, and making certain that therapeutic approaches involving RNAi do not induce unwanted changes in the expression of off-target genes.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

A Novel Method for Accurately Identifying Unknown Genetic Variants Using Mass Spectrometry. *L. Nelson, C. Nelson.* GeneMass Genetics, Salt Lake City, UT.

Linkage mapping may roughly locate a disease-causing gene to within a region of typically 1-2 million base pairs. That region may contain 10 to 100 different candidate genes. To pinpoint the exact disease-associated gene, each gene in that region must be comprehensively analyzed for possible genetic variants. Usually, this screening process is accomplished by DNA sequencing of the entire coding region of each gene, a labor intensive method, especially with regard to data interpretation. There is a growing demand in the genetics community for better technology to accurately and efficiently identify new mutations. Mass spectrometry provides an excellent tool for analysis of genetic variants. Two inherent limitations of mass spectrometry, ability to analyze large molecules and reduced resolution due to broad isotopic distribution peaks, have made it difficult to use mass spectrometry as a competitive technology for screening genes for genetic variants. A method is described that allows the analysis of large PCR products for the detection of small genetic variants.

Using this method, single-base substitutions can be mass spectrometrically resolved in PCR products up to 300 nucleotides. The entire coding region of a gene is analyzed in large segments using PCR and ESI/MS, in which PCR products are generated as monoisotopic (i.e. >99.95% isotopic purity for all elements) using monoisotopic dNTPs. This monoisotopic incorporation yields a significant resolution benefit by greatly narrowing the broad isotope distribution that is normally inherent for large DNA and other biomolecules. As a result, small genetic changes can be easily determined in large PCR products. Additionally, the narrower isotope distribution increases sensitivity and mass accuracy. Several examples of this methodology will be shown. Genes have on average 6-7 exons each consisting on average of 250 to 300 nucleotides. This means that the entire coding region of a gene can be screened in 6-7 monoisotopic PCR reactions, or in only 3-4 reactions with multiplexing. As such, with one mass spectrometer as many as 100 genes could be screened per day.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Generation of a Genome-Wide Library of Regulatory Sequences through the use of a Novel DNAse Hypersensitive Site Cloning Procedure. *G. Crawford[1], I. Holt[1], D. Tai[1], NISC[2], E. Green[1], T. Wolfsberg[1], F. Collins[1]*. 1) NHGRI, NIH, Bethesda, MD; 2) NIH Intramural Sequencing Center, Gaithersburg, MD.

Sequence analysis of the human genome has identified approximately 30,000 coding genes, but little is known about how most of these genes are regulated. Mapping DNAse hypersensitive (HS) sites has been the best method for identifying promoters, enhancers, repressors, and insulators of individual genes. A novel protocol was developed to generate a genome-wide library of regulatory sequences by cloning DNAse HS sites. Genomic DNA from intact nuclei was digested with DNAse at concentrations optimized for known DNAse HS sites, blunt ended, digested to completion with common restriction enzymes, cloned, and sequenced from the blunt end. Over 6000 clones were sequenced from a DNAse HS library generated from quiescent primary human CD4+T cells, and sequences were aligned to the human genome. Our analysis revealed a greater than 10 fold enrichment of sequences upstream of genes when compared to 1000 random in silico libraries, indicating significant enrichment for regions known to contain promoter elements. We also have detected a 10 fold enrichment for sequences that map to CpG islands, which contain promoters of housekeeping genes. Comparative genomics has revealed that the DNAse HS library is significantly enriched for regions that align with the mouse genome, indicating enrichment for evolutionarily conserved regions. Using microarray technology, we have also shown that our library aligns nearby to genes that have detectable transcripts in CD4+ T cells, which reveals that active regions of the genome are being selected. Furthermore, robust identification of valid regulatory elements can be achieved by repeated observation of the same sequence. We have identified and verified thirteen pairs of DNAse HS sites that map to within 700 bp of each other. Surprisingly, a few of these validated DNAse HS sites are greater than 300 kb from any known gene. This procedure, which can be applied to any cell line or tissue, will be useful in identifying regulatory elements controlling global expression differences that delineate tissue types, stages of development, and disease susceptibility.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 233 from 2003 ASHG Annual Meeting

Assessment of the Clinical Readiness of Array-CGH: A Perspective Report. *R.P. Ketterling[1], B.M. Shearer[1], R.C. Locker[2], N. Dzidic[2], M. bin Khalifa[3], M.S. Mohammed[2].* 1) Dept Lab Medicine & Pathology, Mayo Clinic, Rochester, MN; 2) Spectral Genomics, Inc., Houston, TX; 3) Genechip Ltd., London, United Kingdom.

Few new technologies in clinical cytogenetics have generated such excitement as that of microarray-based comparative genomic hybridization (array-CGH). In part, the lures of this approach lay in its amenability to automation, reduced sample turn around times and independence of metaphase preparation and resolution. However, in the absence of the pictorial interpretability of a karyotype, the DNA copy number ratio profiles generated by array-CGH may preclude the correct identification and interpretation of certain chromosomal rearrangements, in particular, mosaic chromosome gains and losses and complex chromosome translocations. The objectives of the current study were to elucidate practical applications for array-CGH technology versus traditional cytogenetic methodologies, and to establish guidelines for clinical adoption. Our conclusions and insights were drawn from a large consortium of array-CGH data collected over the last two years. The results confirm a high concordance (>95%) of array-CGH data with examples of simple microdeletions/duplications, monosomies and trisomies. Mosaicism for autosomal chromosomes was consistently identified at levels of 20-25%, while this sensitivity was diminished for the sex chromosomes. Array-CGH data was informative in some samples where routine G-banding was uninformative or in retrospect, erroneously interpreted. In contrast, a few samples demonstrated the correct DNA copy number by array-CGH but incorrect karyotypic context. Additionally, 20% of samples had apparently benign, repeatable single clone aberrations, which likely represent genomic variants/polymorphisms. While the collective results of this study clearly substantiate the potential of array-CGH, it also illustrates the need for standardized data presentation and interpretation and the judicious incorporation of array-CGH into the repertoire of genetic diagnostic tools.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 234 from 2003 ASHG Annual Meeting

High-resolution array CGH: A validation study for the detection of submicroscopic deletions in DiGeorge and velo-cardio-facial syndromes. *D.G. Albertson[1,2], K.A. Rauen[1,3], P.D. Cotter[3,4,5], R. Segraves[1], T.A. Tokuyasu[1], J. Harris[3], M.E. Elder[3], M. Gonzales[3], D. Pinkel[1,2].* 1) Comprehensive Cancer Center, UC San Francisco, CA; 2) Laboratory Medicine, UC San Francisco, CA; 3) Pediatrics, UC San Francisco, CA; 4) Childrens Hospital and Research Center at Oakland, CA; 5) Division of Medical Genetics, USLabs.

Array-based comparative genomic hybridization (array CGH) allows high-throughput, high-resolution genome scanning for both constitutional and somatic copy number changes. We have used a microarray consisting of 2464 genomic clones providing a genome scanning resolution of approximately 1.4 Mb on average. To assess the application of this array to accurately detect single copy number changes affecting a single clone, we examined 44 patients who were either known to have a single-copy submicroscopic deletion in the DiGeorge critical region (DGCR) on chromosome 22q11.2 or who were undergoing molecular testing for the deletion due to phenotypic abnormalities. All patients had a normal karyotype. Molecular cytogenetic testing for a deletion in the DGCR was performed by FISH. Twenty-six of the 44 samples had a single copy loss of the TUPLE 1 BAC clone. Array CGH was performed on each patient and the results were 100% concordant with standard FISH analyses in detecting the deletion of the TUPLE 1 BAC, thus validating the use of array CGH as a rapid and efficient means to accurately detect single-copy, submicroscopic deletions. The array analysis employed a uniform set of objective criteria to the images and resulting ratio data. In addition to the accurate detection of the DGCR deletion, on average, there were a few other clones per case that had a ratio indicating the possibility of an abnormality (not including clones that show frequent copy number polymorphism in the human population). These abnormal ratios may represent true copy number changes that contribute to the phenotype of the patient, rare copy number polymorphisms or false positive indications of abnormality. We are continuing the development of the analytical criteria to increase the confidence with which measured ratio changes accurately reflect copy number changes in the genome.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 235 from 2003 ASHG Annual Meeting

Clinical validity of a cis-acting modifier of disease penetrance for the common 5T variant in the *CFTR* gene. *J. Groman, T. Hefferon, G. Cutting, The IVS8-5T International Study Group.* McKusick-Nathans Inst Gen Med, Johns Hopkins Sch Medicine, Baltimore, MD.

Incompletely penetrant mutations can obscure identification of disease associations and cause difficulty in diagnosis and genetic counseling. The IVS8-5T mutation of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene is an incompletely penetrant mutation found in approximately 10% of the general population. When found in trans with a severe *CFTR* mutation, the IVS8-5T mutation can result in nonclassic cystic fibrosis, male infertility, or a normal phenotype. To assess the effect of an adjacent, variable TG tract upon penetrance of the IVS8-5T variant, we analyzed DNA samples from 27 unaffected individuals (fertile men), 98 patients with CBAVD, and 9 patients with nonclassic CF. Each of the 134 individuals in this study had a severe CF mutation on one CFTR gene, and the IVS8-5T on the other. 78% (21 of 27) of unaffected individuals had the IVS8-5T adjacent to 11 TG repeats, compared with 9% (10 of 107) of affected individuals. Conversely, 91% (97 of 107) of affected individuals had 12 or 13 TG repeats, versus only 22% (6 of 27) of unaffected individuals ($p < 0.00001$). Therefore, IVS8-5T alleles adjacent to longer TG tracts are substantially more likely to be associated with an abnormal phenotype than IVS8-5T alleles adjacent to the short TG tract (odds ratio= 34.0, 95%CI = 11.1-103.7, $p < 0.00001$). The association between TG tract length and disease penetrance demonstrated in this study is consistent with molecular studies correlating TG tract length with aberrant *CFTR* splicing, and validates clinical use of TG tract testing in the evaluation of patients with IVS8-5T. Knowledge of TG tract length will allow for more accurate counseling of patients positive for the IVS8-5T mutation. Identification and incorporation of genetic tests for other cis acting modifiers of incompletely penetrant mutations may be required to provide accurate genetic testing and counseling for these variants.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 236 from 2003 ASHG Annual Meeting

Variants in the Mannose Binding Lectin gene modify survival of cystic fibrosis patients. *K. Buranawuti*[1], *S. Cheng*[4], *C. Merlo*[2], *P. L. Zeitlin*[3], *B. J. Rosenstein*[3], *P. J. Mogayzel*[3], *X. Wang*[1,3], *M. P. Boyle*[2], *G. R. Cutting*[1,2,3]. 1) McKusick-Nathans Inst Gen Med, Johns Hopkins; 2) Dept of Medicine, Johns Hopkins; 3) Dept of Pediatrics, Johns Hopkins; 4) Roche Molecular Systems Inc.

Cystic Fibrosis (CF) patients with the same mutations in the CFTR gene can differ widely in survival suggesting other factors play a substantial role in mortality. Recently, it was demonstrated that variation in the level of mannose binding lectin (MBL), a component of innate immunity, correlates with lung function of CF patients. Since lung disease is the cause of death in 90% of CF patients, it has been suggested that MBL variation influences survival of CF patients. To test the hypothesis, we compared the distribution of MBL genotypes (A/A, A/O, and O/O) among 6 groups recruited from the same region; 102 children (<17 y.o.) and 115 adults with CF, 37 adult CF patients who had died from lung disease and/or received a lung transplant, 96 children (<17 y.o.) and 142 adults who are healthy, and 179 non-CF adults with chronic rhinosinusitis. Individuals bearing the O/O genotype associated with very low MBL levels were much less frequent among adult CF patients than children with CF (1.7% vs 8.8%; Fishers exact p=0.017). Indeed, the frequency of the O/O genotype was lower in adults with CF than all groups. On the other hand, the O/O genotype was much higher in adult CF patients who had died or had a lung transplant than in surviving adult CF patients (13.5% vs 1.7%; p=0.008). MBL genotype frequencies were similar among children with CF, healthy children, healthy adults and non-CF adults with chronic rhinosinusitis. Furthermore, there were no differences in the genotype frequencies of TNF (-308) or TNF (252, T26N) among the 3 groups of CF patients. Logistic regression analysis reveals that the O/O genotype is associated with a relative risk of 8.83 (95%C.I. =1.6-47.7, p=0.01) for decreased survival compared to A/A and A/O. The survival effect appears to be independent of CFTR genotype since O/O confers a similar risk of 7.63 (95%C.I. =1.4-42.6, p=0.02) among F508/F508. Together, these observations demonstrate the MBL O/O genotype is a common heritable trait that reduces survival of CF patients.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

***ADAM33* polymorphisms predict early-life lung function: a population based cohort study.** *S. John[1], F. Jury[1], A. Custovic[2], J. Holloway[3], A. Woodcock[2], J. Cakebread[3], L. Lowe[2], W. Ollier[1], S. Holgate[3], A. Simpson[2].* 1) CIGMR, Univ Manchester, Manchester, UK; 2) North West Lung Centre, Univ Manchester, UK; 3) Infection, Inflammation and Repair Division, Univ Southampton, UK.

The recent identification of a putative asthma susceptibility gene underlying abnormalities in bronchial hyperresponsiveness (*ADAM33*-a disintegrin and metalloprotease 33) emphasises the possibility that a primary end-organ determinant, rather than the systemic immune response underlies the clinical expression of asthma. Since the majority of asthma has its origin in early life in association with disordered lung function that tracks to persistent disease in later life, it is important to elucidate factors influencing lung function in early life. While standard lung function cannot be measured in pre-school children, plethysmographic measure of specific airway resistance (sRaw) has recently been shown to be feasible. We have analysed 17 SNPs spanning 11Kb of the *ADAM33* gene in 302 children from a prospective birth cohort in which sRaw was completed at age 3 years. We showed a significant association between sRaw and 6 single nucleotide polymorphisms (SNPs) in *ADAM33*. In all cases it was the rare allele that was associated with a higher sRaw value and therefore, poor lung function. When compared to children homozygous for the common allele, carriers of the rare allele had higher sRaw values for SNPs: V-1 (p = 0.024), Q-1 (p = 0.023), ST+7 (p = 0.007) and F+1 (p = 0.001). The association with F+1 was of interest as there was a clear gene dosage effect with mean sRaw being 7% higher in heterozygotes and 12% higher in children homozygous for the rare allele than in the wild type. Children homozygous for the rare allele of T1 (p = 0.001) and T2 (p = 0.003) also had significantly higher sRaw values. Using linear regression analysis, we demonstrated that F+1 was the strongest main effect, V-1, Q-1 and ST+7 had no additional independent effect, whereas the effects at T1 and T2 were independent of F+1. These data support the hypothesis that poor early-life lung function is in part a genetically determined trait involving *ADAM33* that may increase the risk of chronic asthma.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Positional Cloning of Asthma/Atopy Genes in the Interferon Gene Cluster. *A. Chan, D. Newman, C. Ober.* Dept Human Genetics, Univ Chicago, Chicago, IL.

Asthma and atopy are related complex disorders. A genome-wide screen for asthma and atopy susceptibility alleles conducted in the Hutterites, a founder population of European descent, showed evidence for linkage and association with a marker in the type I interferon (IFN) gene cluster on chromosome 9p21 (IFNA STRP, TDT p = 2.5 x 10^{-5}); (Ober et al 2000 AJHG 67:1154-62). The goal of this study was to identify the IFN gene(s) within the cluster that is(are) responsible for this association. The IFN gene cluster spans approximately 400 kb and includes 15 genes and 13 pseudogenes, all comprised of a single exon. We screened 1500 bp of flanking sequences of each functional gene in 12 Hutterites representing all IFNA genotypes, and identified 50 SNPs and 7 indels, including at least one polymorphism flanking each gene. These SNPs defined 20 unique patterns across the region. 27 of these polymorphisms, as well as 6 verified SNPs (3 exonic, 3 flanking) from public databases, were genotyped in 693 Hutterites. Individual SNPs and pairwise combinations of SNPs were examined by the TDT. Sixteen two-locus haplotypes, each of which included SNP IFNA13-1352 were associated with bronchial hyperresponsivness to methacholine (P = 0.026 to 0.00062). In addition, 8 two-locus haplotypes, each of which included SNP IFNB-946, were associated with allergic sensitization to molds (P = 0.04 to 0.0039), and 11 two-locus haplotypes, each of which included SNP INFA16-1995, were associated with allergic sensitization to cockroach (P = 0.04 to 0.0034). We are currently screening the exons of these three genes for additional variation. These results suggest that multiple type I interferon genes are involved in asthma and atopy susceptibility, and that the susceptibility variants reside on haplotypes that show long-range linkage disequilibrium. Supported by HL56399.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 239 from 2003 ASHG Annual Meeting

LD MAPPING AND HAPLOTYPE ANALYSIS AT 7q31-7q34 IMPLICATE MULTIPLE GENES ASSOCIATED WITH BODY MASS INDEX IN THE NHLBI FAMILY HEART STUDY. *Y. Jiang*[1], *J.B. Wilk*[1], *I. Borecki*[2], *S. Williamson*[1], *A.L. DeStefano*[1], *M. Province*[2], *R.H. Myers*[1]. 1) Dept Neurology, Boston Univ Sch Medicine, Boston, MA; 2) Division of Biostatistics, Washington University School of Medicine, Saint Louis, MO.

Multipoint linkage analysis revealed a LOD score of 4.7 for Body Mass Index (BMI) in the National Heart, Lung, and Blood Institute (NHLBI) Family Heart Study at 7q31-34. Leptin (LEP), located at 7q32, is the most prominent candidate gene in this region. While mutations in the leptin gene are known to lead to rare obese syndromes in humans, no mutations have been unambiguously identified in the LEP gene in patients with common multifactorial obesity. To investigate whether LEP and/or a larger 10Mb region harboring other susceptibility genes may contribute to the linkage signal, we conducted high throughput SNP genotyping on 640 individuals from 80 families demonstrating linkage to this region. Family-based tests of association were implemented by TRANSMIT and FBAT using about 200 single-nucleotide polymorphisms (SNPs) across 10Mb from D7S2847 to D7S2560. Evidence for association to BMI was observed in three different genes in both quantitative trait and dichotomous phenotypes (P =. 0017 for LEP, P = .0003 for PODXL and P = .0022 for CALD1 in single SNP TRANSMIT analysis). We investigated haplotype association for these regions, selecting SNPs for a haplotype from different haplotype blocks defined using LD mapping methods. In the LEP region, haplotype analysis revealed significant global P values, of P=.0002 for a three-SNP haplotype and P=.00001 for a five-SNP haplotype. A common five-SNP haplotype extending in the 5 region of LEP with a frequency of 58.4% is over transmitted from parents to affected offspring. That these three regions are not in LD with one another suggests that there may be more than one gene in the 7q317q34 region influencing BMI.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 240 from 2003 ASHG Annual Meeting

Body mass index modifies the association of interleukin 6 genotype with insulin resistance in men: the Framingham Offspring Study. *A. Herbert[1,2], C. Liu[2], S. Karamohamed[2], S. Liu[2], P.W.F. Wilson[4], J.A. Meigs[5], L.A. Cupples[3]*. 1) Department of Genetics & Genomics, Boston University School of Medicine, Boston, MA; 2) Framingham Heart Study Genetics Laboratory, Department of Neurology, Boston University School of Medicine, Boston, MA; 3) Department of Biostatistics, Boston University School of Public Health, Boston, MA; 4) Department of Medicine, Boston University School of Medicine, Boston, MA; 5) General Internal Medicine and Clinical Epidemiology Units, General Medicine Division, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA.

The interleukin 6 (IL6) gene -174 promoter polymorphism has been proposed as a risk factor for type 2 diabetes mellitus, but results are conflicting. A major source of IL6 in resting individuals is white fat, raising the possibility that body mass index (BMI) may modify effects of the -174 IL6 polymorphism on insulin resistance and diabetes risk. We examined a community-based cohort study of 1526 adults (mean age 55.6 years, 753 men) who participated in the Framingham Offspring Study during the 1991-1995 examinations. Significant interactions between IL6 genotype and BMI were found in men for all measures of insulin resistance (HOMA-IR, Gutt ISI 0,120, McAuley Index) ($p<0.0001$), with obese homozygotes for the minor C allele being most resistant. Among men with the CC genotype (f=0.16), increasing BMI was associated with a higher prevalence of type 2 diabetes (DM2) (OR per unit increase in BMI =1.30 (95% CI 1.11-1.50)) but not among those with the GG (f=0.34, OR=1.10 95% CI 0.98-1.22) or GC genotypes (f=0.50, OR=1.05 95% CI 0.97-1.14). Obesity thus affects men with a CC genotype differently from those with a G allele, which in this context appears protective against BMI induced DM2. This study underscores the role of gene-environment interactions in complex diseases such as DM2.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Common non-coding SNPs near the *Hepatocyte Nuclear Factor-4 Alpha* gene are associated with type 2 diabetes.

K.L. Mohlke[1], K. Silander[1], E.C. Peck[1], L.J. Scott[2], P.E. Hollstein[1], A.D. Skol[2], C. Li[2], P.S. Chines[1], T.T. Valle[3], R.N. Bergman[4], J. Tuomilehto[3], R.M. Watanabe[4], M. Boehnke[2], F.S. Collins[1]. 1) NHGRI, Bethesda, MD; 2) U. Michigan, Ann Arbor, MI; 3) National Public Health Institute, Helsinki, Finland; 4) U. Southern California, Los Angeles, CA.

The Finland-United States Investigation of NIDDM genetics (FUSION) study aims to positionally clone variants that predispose to type 2 diabetes (T2D). A genome scan using 495 Finnish affected sibling pair families yielded a LOD score of 2.32 at 69 cM on chromosome 20q12-13.3; linkage in this region has been observed in 7 other populations. To fine-map the 13 cM (7.4 Mb) 1-LOD support interval, we are genotyping SNPs on case and control DNA pools. Among the first 291 SNPs, we selected 32 to confirm by individually genotyping 793 T2D cases (one from each family) and 413 controls. The most strongly associated SNP has a frequency of 0.201 in cases and 0.156 in controls (OR 1.36, p-value .007) and is located 1 kb downstream of an alternative transcription site P2 for the *Hepatocyte Nuclear Factor-4 Alpha* (*HNF4A*) gene. We genotyped 39 additional nearby SNPs, including 19 selected from a haplotype map (kindly provided by P. Deloukas, Sanger Institute). The strongest association is observed with the original SNP and one located 5.3 kb away that is in complete linkage disequilibrium (LD). These SNPs are located in a block of LD that extends >143 kb upstream of *HNF4A*. We observed greater linkage evidence in families where the genotyped sibling has at least one risk allele compared to families where the genotyped sibling has no risk alleles (MLS=1.93 vs 0.17). Mutations in *HNF4A* are known to cause maturity-onset diabetes of the young (MODY), a monogenic dominantly-inherited form of diabetes, but no previously identified variants within *HNF4A* have been associated with T2D. Significantly, a SNP in complete LD with the 2 reported here was detected independently and found to be associated with T2D (p-value .003) in Ashkenazim (L. Love-Gregory and M.A. Permutt, personal communication). These data suggest that defects in unknown *HNF4A* regulatory elements near the P2 promoter may increase susceptibility to T2D.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

A Common haplotype in the glucokinase gene is associated with increased fasting glucose and altered birth weight. *T.M. Frayling[1], M. Weedon[1], B. Shields[1], B. Knight[1], T. Wilkin[1], L. Voss[1], B. Metcalf[1], G. Davey-Smith[2], Y. Ben-Shlomo[2], D. Leon[3], V. Mann[3], R. Mohsen[4], A.C. Syvanen[4], T. Axelsson[4], L. Byberg[4], A.T. Hattersley[1]*. 1) Peninsula Medical School, Exeter&PLymouth , UK; 2) Department of Social Medicine, Bristol, UK; 3) London School of Hygiene and Tropical Medicine, UK; 4) Uppsala University, Sweden.

Rare mutations in the glucokinase gene (GCK) cause hyperglycaemia and alter birthweight. The effects of common GCK variants are not known. We sequenced the coding region and 13 dbSNPs within intron and 5 regions of GCK in 24 individuals. Pairwise LD and haplotypes were computed. For association analyses we genotyped a G/A variant at postion -30 (rs1799884; A allele frequency 18 percent) in part of the islet-cell promoter conserved between human and mouse. Four European cohorts were used: 459 Exeter trios; 160 Plymouth trios; 431 Uppsala mother and 2 children families and 636 Barry Caerphilly Growth (BCG) cohort individuals. Nine SNPs were identified, with rare allele frequencies ranging from 7-40 percent. Pairwise LD estimates were consistent with the presence of two blocks, with weak LD occuring between 5 and intron 1 SNPs. The -30G/A variant defined one of 3 haplotypes that together accounted for 86 percent of all haplotypes in the 5 region. Across all studies fasting glucose was increased by 0.08 mmol (p = 0.00008) for GA/AA versus GG subjects (Exeter, p=0.008; Plymouth, p=0.036; and BCG, p =0.03). Birth weight was increased by a mean of 251g in AA v GG mothers (all cohorts p=0.002). Mothers carrying the A allele had offspring 65g heavier compared to those that did not (combined p=0.03). Offspring of GG mothers inheriting a paternal A allele were lighter by 122g compared to those inheriting a paternal G allele (combined p = 0.01) and in the Uppsala discordant sib analysis there was a trend to the child with the polymorphism being 93g lighter at birth than the sibling with the mutation (p=0.15). This is the first common genetic variant to be associated with fasting blood glucose, and provides evidence that birth weight can be altered by common as well as rare genetic variants in both mother and fetus.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Genetic variation at the POMC locus partially defines the chromosome 2p human obesity QTL. *S.A. Cole*[1], *A.G. Comuzzie*[1], *T. Dyer*[1], *M. Britten*[1], *M. Bounpheng*[2], *D. Rines*[2], *L. Almasy*[1], *J. Williams*[1], *J. Blangero*[1]. 1) SW Foundation for Biomedical Research, San Antonio, TX; 2) Accelerated Genomics, Inc., San Antonio, TX.

The San Antonio Family Heart Study (SAFHS) was the first to identify a human obesity QTL on chr 2p that influences leptin levels. A strong positional candidate in the linkage region is the gene for proopiomelanocortin (POMC). By means of two-dimensional gene scanning (TDGS) using OptiScanHT technology, we analyzed 35 PCR fragments from the POMC gene region for polymorphism in 414 SAFHS subjects, and identified 25 SNPs. There was surprisingly little linkage disequilibrium in this gene suggesting that most markers have independent information and need to be typed directly. We employed Bayesian quantitative trait nucleotide (BQTN) analysis to determine the most likely SNPs influencing leptin levels. Six SNPs were at least marginally associated with leptin levels. BQTN analysis was used to evaluate all possible additive models of gene action after pruning of redundant SNP sets. Model averaging was used to estimate posterior probabilities of SNP functionality and to eliminate the multiple testing problem. We found strong evidence of putative functionality for 4 different SNPs. Linkage analysis was used to assess whether these SNPs could completely account for the observed linkage. Including these SNPs in the model reduced the LOD score from 5.13 to 2.39, suggesting that more than half of the linkage signal can be explained by POMC SNPs. Together these results suggest that POMC variation contributes to the chr 2 obesity QTL. However, it also seems likely that there are other genes in the region contributing to the QTL since the residual LOD score is still significant. Given the high level of replication for this QTL, this hypothesis is reasonable. Interestingly, 3 of the associated POMC SNPs had frequencies of 0.06 and were effectively uncorrelated with any other markers. Thus, these data also suggest the cumulative importance of multiple rarer variants influencing quantitative trait variation. Therefore, association strategies employing common haplotype blocks would fail for this important gene.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Converging evidence for an essential hypertension susceptibility locus on chromosome 1q. *Y. Chang[1], D. Kim[1], M. Ikeda[1], X. Liu[1], A. Mitchell[1], S. Gillespie[1], M. Kenton[1], A. Weder[2], R. Cooper[3], A. Luke[3], A. Chakravarti[1].* 1) Johns Hopkins University, Baltimore, MD; 2) University of Michigan Hospital, Ann Arbor, MI; 3) Loyola University Medical Center, Maywood, IL.

In order to uncover genes involved in blood pressure regulation, we performed genome-wide linkage analysis on 1,875 individuals (585 families) collected through the GenNet network of the NHLBI Family Blood Pressure Program. We performed variance components linkage analysis using GENEHUNTER2 and SOLAR. Caucasian and African American samples were analyzed separately and jointly, and we used various combinations of sex, age, BMI, and smoking status as covariates. The most significant lod score (3.97) was between diastolic blood pressure and the marker D1S1589 (192 cM) at chromosome 1q25.1. We performed TDT analysis using the same marker data. Affecteds were defined as those on hypertension medication or those with SBP>140 mmHg and/or DBP>90 mmHg. The most significant finding (TDT score= 14.4, P = 0.0002) was an allele of marker D1S1677, located under the chromosome 1 linkage peak. The linkage peak spans D1S1677 to D1S1678 and covers ~43 cM (40 Mb), a region containing more than 200 known genes. Based on expression patterns and known or putative function, we identified 24 candidate genes involved in salt transport, lipid metabolism, renal glomeruluar filtration, and signal transduction. So far we have examined variants in and flanking three such candidate genes, NPHS2, ADORA1, and ATP1B1. We found consistent and significant association between a cluster of SNPs in an approximately 6 Kb region and hypertension status and blood pressure. Our linkage results replicate two previous studies on the genetic basis of hypertension: the NHLBI Family Heart Study and the Finnish Twin Cohort Study. Furthermore, human chromosome 1q21-1q32 is syntenic to a region on rat chromosome 13 that contains multiple blood pressure related QTLs. In summary, chromosome region 1q25-1q32 contains one or more genes that influences blood pressure. Potential involvements in blood pressure regulation of selected candidate genes from this region are being determined by fine-mapping, association studies, and functional analysis.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 245 from 2003 ASHG Annual Meeting

Elastin Gene Mutations in Patients with Cutis Laxa: Genotype-Specific Pulmonary and Vascular Lesions. *Z. Urban[1], M.W. Crepeau[1], Z. Szabo[1], F.M. Pope[2], M.J. Stephan[3], A. Mitchell[4], L.K. Yin[5], E.C. Davis[6], The Cutis Laxa Consortium.* 1) Pacific Biomedical Res Ctr, Univ Hawaii, Honolulu, HI; 2) West Middlesex University Hospital, Isleworth, United Kingdom; 3) Madigan Army Medical Center, Tacoma, WA,; 4) University of Washington, Seattle, WA; 5) Childrens Hospital, Singapore,; 6) McGill University, Montreal, Canada.

Elastin gene (ELN) mutations may cause two distinct disease phenotypes: supravalvular aortic stenosis (SVAS) or cutis laxa (CL). Over 50 ELN mutations have been described in SVAS patients, but only 3 ELN mutations have been reported in patients with CL to date. To define the spectrum of ELN mutations underlying CL, to uncover new genotype-phenotype correlations, and to better understand the molecular mechanism of this disease, we have analyzed 16 unrelated probands with CL for mutations in ELN. A partial, tandem duplication was identified in a family with autosomal dominant CL, hernias and adult-onset chronic obstructive pulmonary disease characterized by bronchiectasis and emphysema. Two singleton individuals with CL were found to carry single nucleotide deletions in exon 30 of ELN (2159delC and 2166delT). One of these mutations (2159delC) was a de novo recurrent mutation. A 25-nucleotide deletion (2112_2136del) was found in a family with mild cutis laxa, hernias and thoracic aortic aneurysms. Functional analysis of 3 of these 4 mutations indicated equal expression of the mutant and normal alleles at the mRNA and protein levels. Mutant-specific antiserum was used to demonstrate secretion and matrix deposition of the duplicated tropoelastin. We conclude that ELN mutations in CL patients lead to the synthesis and incorporation of mutant tropoelastin into the elastic fiber. Mutation-specific presence of lesions associated with CL including emphysema or aortic aneurysms suggests that differences in the mutant polypeptides may have important effects on the phenotype of CL patients.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 246 from 2003 ASHG Annual Meeting

Autosomal recessive Ehlers-Danlos phenotype with severe valvular disease caused by homozygous *COL1A2* premature termination mutations. *A.E. Beck[1], K. Chen[1], U. Schwarze[2], P.H. Byers[2,3], H.E. Hoyme[1]*. 1) Div Genetics, Dept Pediatrics, Stanford University School of Medicine, Stanford, CA; 2) Dept Pathology and; 3) Dept Medicine, University of Washington, Seattle, WA.

The classical form of Ehlers-Danlos syndrome (EDS, types I and II) is an autosomal dominant condition characterized by joint hypermobility and skin hyperextensibility. The majority of these patients have heterozygous mutations in the *COL5A1* or *COL5A2* genes, the protein products of which combine to form type V collagen. We report on a family in which two brothers have severe valvular heart disease in addition to phenotypic signs of classical EDS and homozygous premature termination mutations in the *COL1A2* gene. The proband is a 29-year-old man with hyperextensible joints and cardiac disease. He required surgery for bilateral inguinal hernias, pes planus and calcaneovalgus. He has soft, hyperelastic skin and vertical striae on the flank. He recently required both aortic and mitral valve replacement for severe aortic and mitral regurgitation. His parents (who are second cousins) have no joint hypermobility, scarring or cardiac disease. His 26-year-old brother, however, has significant joint hypermobility and soft skin, and he required aortic valve replacement secondary to aortic regurgitation. Fibroblasts from the proband do not synthesize the pro2(I) chains of type I procollagen. A homozygous G -> T transition mutation (at position 3601 of the coding sequence) was found in the *COL1A2* gene that created a premature termination codon in exon 50 (E1201X) of both brothers. The mRNA from these alleles was destabilized by the nonsense-mediated decay mechanisms and was in extremely low abundance in the cell. When evaluating patients with a classical Ehlers-Danlos-like phenotype and significant valvular disease, especially in those pedigrees suggestive of autosomal recessive inheritance, homozygous mutations in the *COL1A2* gene must be considered.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 247 from 2003 ASHG Annual Meeting

Characterization of the phenotype associated with familial thoracic aortic aneurysms and dissections. *E.M. Gutter[1], V.T. Fadulu[1], S.N. Hasham[1], T.M. King[1], M.C. Willing[2], A.C. Muilenburg[2], T. Chin[1], D.M. Milewicz[1]*. 1) Internal Medicine, Univ of Texas Medical School; 2) Pediatrics, Univ of Iowa.

Familial thoracic aortic aneurysms and dissections (TAAD) is an adult onset autosomal dominant disorder characterized by ascending thoracic aortic disease and is associated with variable expression and decreased penetrance. Two loci have been mapped for the condition: TAAD1 on 5q13-14 and TAAD2 on 3p24-25. This study aimed to describe and compare phenotypic features of TAAD using 70 individuals who inherited the affected haplotype from six large families: 24 individuals from four families linked to TAAD1 and 46 individuals from two families linked to TAAD2. The disease was highly penetrant; 96% of individuals with the affected haplotype had evidence of aortic disease. All nonpenetrant individuals were female, with three of 23 females not showing evidence of aortic disease. Features of aortic disease were similar between the two loci, however TAAD1 was associated with a higher occurrence of aortic dissections ($p=0.023$) and TAAD2 was associated with a higher occurrence of skeletal anomalies ($p=0.016$). There were more males (47) than females (23) who inherited the affected haplotype. To test for a skewed offspring ratio in the families, the gender of offspring from individuals with the affected haplotype was determined. Surprisingly, individuals who inherited the defective gene at the TAAD2 locus were more likely to have male offspring than female no matter the sex of the affected parent (98 children, 61 males and 37 females, $p=0.004$), whereas individuals linked to TAAD1 were not (87 children, 46 males and 41 females, $p=0.33$). Interestingly, anticipation in the age of onset of aortic disease was found for both loci; the age of onset for generation I was 61 years, the age of onset for generation II was 45 years, and the age of onset for generation III was 22 years ($p<0.001$). Therefore, familial TAAD demonstrates surprising clinical parallels with another adult onset, autosomal dominant disorder affecting the vascular system, familial primary pulmonary hypertension, in which there is anticipation, decreased penetrance in males, and skewed offspring ratio favoring females.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 248 from 2003 ASHG Annual Meeting

Mutations in *SMAD4* cause a combined Hereditary Hemorrhagic Telangiectasia-Juvenile Polyposis syndrome.

C.J. Gallione[1], G.M. Repetto[2], E. Legius[3], A.K. Rustgi[4], S.L. Schelley[5], M. Dunlop[6], G. Mitchell[7], E. Drouin[7], C.J.J. Westermann[8], D.A. Marchuk[1]. 1) Dept. of Molecular Genetics and Microbiology, Duke Univ., Durham, NC; 2) Facultad de Ciencias de la Salud, Univ. del Desarrollo-Clinica Alemana and Hosp. Padre Hurtado, Santiago, Chile; 3) Center for Human Genetics, Leuven, Belgium; 4) Dept. of Genetics, Univ. of Pennsylvania, Philadelphia, PA; 5) Dept. of Pediatrics/Genetics, Stanford Univ., Stanford, CA; 6) Dept. of Surgery, MRC Human Genetics Unit, Univ. of Edinburgh, Edinburgh, Scotland; 7) Dept. of Medical Genetics, Ste Justine Hosp., Montreal, Canada; 8) Dept. of Pulmonary Disease, St. Antonius Hosp., Nieuwegein, The Netherlands.

Hereditary Hemorrhagic Telangiectasia (HHT) and Juvenile Polyposis (JP) are two autosomal dominant disorders characterized by distinct and essentially non-overlapping clinical features. HHT is a vascular malformation disorder caused by mutations in either *Endoglin* or *ALK1*. JP, an inherited gastrointestinal malignancy predisposition, is caused by mutations in either *SMAD4* or *BMPR1A*. All four genes encode proteins involved in the TGF- signaling pathway. There are reports of patients and/or families with combined HHT and JP phenotypes, with some speculating that these cases represent a novel syndrome. Nonetheless, the underlying genetic etiology of this association remains unknown. We collected samples from 8 unrelated families/patients segregating both HHT and JP. No mutations were identified in these patients in either the *Endoglin* or *ALK1* genes; instead, all harbored *SMAD4* mutations, including one individual with a *de novo* mutation in *SMAD4*. These data confirm that mutations in *SMAD4* cause this previously unnamed syndrome consisting of both HHT and JP phenotypes. The data also show the existence of a third locus for HHT in this phenotypically unique subset of families. Genetic testing is recommended for patients presenting with either phenotype to reveal those at risk for this syndrome. JP patients harboring *SMAD4* mutations should be screened for the vascular lesions associated with HHT, especially occult arteriovenous malformations in visceral organs that may otherwise present suddenly with serious medical consequences.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Frequency and spectrum of *PTEN* mutations in Lhermitte-Duclos disease, irrespective of family history or syndromic features, suggest this as a pathognomonic feature of Cowden syndrome. *C. Morrison, X.P. Zhou, D. Marsh, M. Maxwell, G. Reifenberger, C. Eng.* Human Cancer Genetics Prog, Ohio State Univ, Columbus.

Lhermitte-Duclos disease (LDD), or dysplastic gangliocytoma of the cerebellum, is a rare hamartomatous lesion resulting in ataxia and, increased intracranial pressure and seizure. LDD can be familial or more commonly sporadic. Based on single case families, it has only recently been suggested that LDD may be associated with Cowden syndrome (CS), an autosomal dominant disorder characterized by multiple hamartomas and by an increased risk of breast, thyroid and endometrial neoplasia. Germline *PTEN* mutations are associated with 80% of CS patients. It remains unclear whether all cases of LDD, even without features of CS, are caused by germline *PTEN* mutation, and whether somatic PTEN mutation can account for sporadic LDD. We obtained paraffin-embedded LDD lesions from 18 unselected, unrelated patients and performed mutational analysis of *PTEN*. Overall, 15 of 18 (83%) samples were found to carry a *PTEN* mutation, irrespective of whether they had features of CS or not. Of note, all 15 with mutations were adult onset and the 3 without mutations were diagnosed at the ages of 1, 3 and 11 years. Germline DNA was available from 6 cases, all 6 had germline *PTEN* mutations. Immunohistochemistry revealed that 75% of LDD samples had complete or partial loss of PTEN expression accompanied by increased phosphorylated Akt in the dysplastic gangliocytoma cells. These data suggest that loss of PTEN function, whether germline or somatic, is sufficient to cause LDD. The high frequency and spectrum of germline *PTEN* mutations in patients ascertained by LDD alone confirm that LDD is an important defining component of CS. Our observations suggest that virtually all adult onset LDD cases have germline *PTEN* mutations, and LDD should be considered a pathognomonic feature of CS. Individuals with LDD, even without apparent CS features, should be counseled as in CS, even without gene testing results. Further, our 3 childhood onset LDD, together with 3 others reported in the literature, all without *PTEN* mutations, suggest that childhood LDD is distinct from adult LDD.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 250 from 2003 ASHG Annual Meeting

Epilepsy, fetal anticonvulsant syndrome, and polymorphisms in MTHFR and SHMT, two folate pathway genes.

J. Dean[1], Z. Robertson[1], S. Yousaf[1], H. Hailey[1], S. Moore[2], P. Turnpenny[3], J. Watson[1], D. Lloyd[4], D. Shaw[1], J. Little[1].
1) Dept Medical Genetics, Medical School, Aberdeen, Scotland, United Kingdom; 2) Medical Genetics Program, Memorial University of Newfoundland, St Johns, Newfoundland, Canada; 3) Clinical Genetics Service, Royal Devon and Exeter Hospital, Exeter, UK; 4) Neonatal Unit, Aberdeen Maternity Hospital, Aberdeen, UK.

Between 6 and 14 % of children born to epileptic mothers taking anticonvulsant drugs have malformations requiring surgery, and up to 20% have delayed development in later childhood. Other clinical features such as facial findings facilitate a diagnosis of a fetal anticonvulsant syndrome (FACS) in many cases. Because some of the malformations associated with FACS are also associated with folic acid deficiency, and polymorphisms in MTHFR, we previously investigated the 677C>T MTHFR polymorphism and showed an association between FACS and the maternal MTHFR genotype. We now provide a preliminary report on an extended study including 276 children born to 181 mothers taking anticonvulsants in pregnancy. Data from these families has been combined with data from the earlier study of families identified through a parent support group. An excess of 677C>T homozygotes was seen in epileptic mothers (p=0.04) compared with non-epileptic controls, but the association of the TT maternal genotype with fetal anticonvulsant syndrome in the child is also confirmed (p=0.02). To investigate folate pathway genes further, genotyping of the 1420C>T polymorphism in SHMT (serine hydroxymethyl transferase) was undertaken. This enzyme may act as a metabolic switch between pathways leading to methylation of DNA and proteins and pathways involved in thymidine synthesis. Although the 1420 polymorphism has been shown to alter plasma homocysteine levels and the distribution of folate derivatives in vivo, no association with epilepsy or fetal anticonvulsant syndrome was found in this study. Interestingly, there was a trend towards an excess of 1420 C>T heterozygotes in mothers of children with FACS associated developmental delay, but this was not statistically significant. The interaction between folate pathways, epilepsy and FACS is complex and requires further study.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 251 from 2003 ASHG Annual Meeting

Congenital Anomalies Following Gestational Statin Exposure. *R. Edison, M. Muenke.* Medical Genetics Branch, DHHS/NHGRI/NIH, Bethesda, MD.

Statin drug use for prevention of cardiac morbidity is increasing among women of child-bearing age. Statins are contraindicated in pregnancy due to potent inhibition of mevalonate and cholesterol biosynthesis, and teratogenicity in animals. Impact on human development is unstudied. Case series data of gestational exposure were examined to assess whether prospective studies seem indicated, and which metabolic/regulatory processes may be implicated.

Methods: Review of all case reports (N=214) from the FDA surveillance database. Exclusion: 1st trimester losses, unknown outcomes. Pregnancy outcomes were categorized: normal, growth anomaly (IUGR), intrauterine demise (IUFD), or structural anomaly.

Results: Of 69 evaluable outcomes, 40 were normal, including all reports of pravastatin (n=20) and fluvastatin exposure (n=3). 4 cases of severe IUGR were reported (all simvastatin), and 4 cases of IUFD (2 simvastatin, 2 lovastatin). 21 structural anomaly reports all followed exposure to a lipophilic statin (7 lovastatin, 9 simvastatin, 4 atorvastatin, 1 cerivastatin). Distinct clusters of malformation were seen: 5 limb reduction defects(LRDs): 2 complex lower LRDs (both intercalary and foot defects, both simvastatin, <1/100K births background); 1 complex upper LRD with intercalary defect and radial aplasia (lovastatin, 1/100K); atretic thumb (lovastatin); intercalary arm truncation (atorvastatin, 1/1M). CNS cases: 2 holoprosencephaly (HPE) (cerivastatin, lovastatin, 1/16,000), aqueductal stenosis (lovastatin), complex open neural tube defect (NTD) with palate agenesis (lovastatin), and NTD (atorvastatin). Total numbers of pregnancies exposed to each drug is unknown; estimates are in the low 1000s for each drug. Most are unreported.

Conclusions: Clusters of rare limb and CNS defects, seen with cholesterol biogenesis disorders or Sonic Hedgehog (SHH) dysfunction suggest a biologic basis to these adverse findings. Controlled prospective studies of inadvertently exposed pregnancies are needed to assess the true level of teratogenic risk associated withprenatal statin exposure.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Molecular Testing for TWIST and FGFR1-3 Mutations in a Cohort of 153 Craniofacial Patients. *A.M. Turner[1], T. Roscioli[2], G. Elakis[2], P.J. Taylor[2], T. Cox[3], E. Haan[3], C. Oley[4], J. McGaughran[4], J. Dixon[5], M. Edwards[6], R. Savarirayan[7], M. Gianoutsos[8], D.J. David[9], M.F. Buckley[2], V. Pospisil[2].* 1) Department of Medical Genetics, Sydney Children's Hospital, Sydney, Australia; 2) SEALS Molecular and Cytogenetics Laboratory, Prince of Wales Hospital, Randwick, NSW, Australia; 3) 3Department of Medical Genetics, Women and Children's Hospital, Adelaide, SA, Australia; 4) Queensland Clinical Genetics Service, Herston, QLD, Australia; 5) Department of Medical Genetics, Wellington, New Zealand; 6) Department of Medical Genetics, Newcastle, NSW, Australia; 7) Victorian Clinical Genetics Service, Melbourne, VIC, Australia; 8) Craniofacial Unit, Sydney Children's Hospital, Randwick, NSW, Australia; 9) Australian Craniofacial Unit, Women and Children's Hospital, Adelaide, SA, Australia.

Craniofacial disorders are a common presenting feature in clinical genetics practice, with the overall incidence of craniosynostosis being 1 in 2500. 30-70% of a subgroup of syndromic craniosynostoses have been described in the literature to be caused by fibroblast growth factor receptor (FGFR) mutations. These mutations are clustered in gene hotspots, the FGFR1-3 II-III Immunoglobulin-like linker region and the third immunoglobulin-like loop of FGFR2. We present a cohort of 153 individuals referred for detection of mutations in FGFR1-3 and TWIST by DHPLC screening and gene sequencing. Mutations were found in 37% of the probands, the majority having been previously described in mutation bearing exons. In addition, four novel sequence variants were detected in FGFR2 (Lys367Glu, Ala266Pro, Tyr340Asn) and FGFR3 (Pro283Thr) in individuals with a Crouzon (FGFR2) and bi-coronal (FGFR3) phenotype. The clinical features that were predictive for mutation detection included multisutural synostosis and a phenotype consistent with the known FGFR/TWIST-related syndromes, particularly Crouzon, Pfeiffer and Apert syndromes. Targeting gene hot spot areas for mutation analysis has proven to be a useful strategy to maximize the success of molecular diagnosis of the craniofacial disorders.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Molecular characterization of Pfeiffer syndrome: Implications for prognosis and genetic counseling. *D.M. McDonald-McGinn[1], R. DeBerardinis[1], S. Bartlett[1], L. Whitaker[1], I. Jacobs[1], K. Gripp[2], C. Stolle[1], A.O.M. Wilkie[3], M. Muenke[4], E.H. Zackai[1]*. 1) Children's Hospital of Phialdelphia and the University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Alfred I. duPont, Wilmington, DE; 3) Weatherall Institute of Molecular Medicine, Oxford, England; 4) National Institutes of Health, Bethesda, MD.

Cohen (1993) classified Pfeiffer syndrome (PS) based on severity with PS Type I being mildly affected, Type II with a cloverleaf skull & Type III without cloverleaf skull. We identified mutations in 11 patients (9 probands & 2 parents) with PS: 1 mildly affected child with a P252R mutation in FGFR1; a typically affected parent & child with normal intelligence & a splice mutation in exon IIIc of FGFR2; an atypical parent & child with a K641R mutation in FGFR2; and 6 severely affected children (1 diagnosed in utero) with mutations in FGFR2-W290C(1), C342S(2), S351C(3). Patients with the latter mutations, previously described as PS Types II or III, can have significant & life threatening non-craniofacial anomalies, including tracheal, gastrointestinal, CNS and vertebral fusion defects. 2 patients (W290C and S351C) with tracheal sleeves succumbed in the first year of life (9 months & 15 days). Two other patients(C342S) also had a tracheal sleeve, 1 requiring a tracheotomy at 11 months of age. All children with the severe mutations who survived the newborn period had significant developmental delay/mental retardation. Moreover, all 3 patients with the S351C mutation had abnormal MRI scans including polymicrogyria, agenesis of the corpus callosum(2) & absent septum pellucidum. Two of these 3 children succumbed in late childhood (7 & 15 years). Both had profound mental retardation. Thus, we suggest that mutation identification, which is available both in utero & in the newborn period with a rapid turn around time, is helpful in guiding medical management decisions following the definition of abnormalities in the neonatal airway, vertebrae, gastrointestinal and central nervous systems. Furthermore, this information will assist in providing counseling regarding prognosis including long-term survival, quality of life, & recurrence risk.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Adult adaptive functioning in 22q11 Deletion Syndrome. *E. Chow[1,2], M. Watson[1], V. Wong[1], A.S. Bassett[1,2].* 1) Centre for Addiction and Mental Health, Toronto, ON, Canada; 2) Dept Psychiatry, Univ Toronto, Toronto, ON, Canada.

Background: 22q11 Deletion Syndrome (22qDS) is a common microdeletion syndrome associated with an approximately 40% prevalence of mental retardation. Up to 30% of 22qDS adults may develop schizophrenia (SZ). Cognitive abilities of children and adults with 22qDS have been described in several reports, but the level of adaptive functioning in 22qDS adults has not been reported. Method: The Vineland Adaptive Behavior Scales were completed on 43 adults (mean age=27.4y, SD=7.8y; 19 M 24 F) by direct interview of the subject and/or their primary caretaker. Nineteen subjects suffered from DSM-IV SZ (22qDS-SZ) and 24 subjects had no history of psychosis (22qDS-NP). IQ scores were obtained using WAIS-R or WAIS-III. Results: Overall, 22qDS subjects had a mean IQ of 69.1 (SD=8.4) and on average performed at an age-equivalent level of 10y 7m (communication), 13y 5m (daily living skills), 10y 11m (socialization), 5y 9m (motor skills), and 11y 5m (adaptive behaviour composite score). The 22qDS-NP and 22qDS-SZ subjects did not differ significantly in sex distribution or mean IQ, but the 22qDS-SZ subjects were significantly older (mean age 30.8y vs 24.7y, p=0.009). Compared to the 22qDS-NP group, 22qDS-SZ group functioned at a similar age-equivalent level in the communication and motor skills domains, but at a significantly lower level in the daily living skills (mean 11y 0m vs 15 y 5m, p=0.0003), socialization (mean 8y 7m vs 12y 9m, p=0.0007), and in adaptive behaviour composite (mean 9y 8m vs 12y 11m, p=0.003) even after correction for multiple testing. Analyses of the subdomain scores revealed a significantly lower level of functioning in 22qDS-SZ subjects in the receptive communication subdomain, the personal and community daily living skills subdomains, and the interpersonal relationships and play and leisure time socialization subdomains. Conclusions: Overall, 22qDS adults performed only at a pre-teen level in most domains of adaptive behaviour, and those with SZ performed even worse in domains that are important to social functioning.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Cystic fibrosis prenatal screening of over 50,000 members in a large HMO. Program update with an emphasis on the outcomes of high-risk pregnancies, reduction in the incidence of CF, and clinical utility. *D. Witt, C. Wold, E. Louie, P. Goonewardena.* Genetics Dept., Kaiser Permanente Northern CA, San Jose, CA.

This report gives an update on the first 4 years of operation of a CF prenatal screening program in the largest prepaid group medical practice in the U.S. The discussion will include identification rates of carriers, high-risk couples, and specific CFTR mutations. It will focus on decision-making in, and outcomes of, high-risk pregnancies including the options of prenatal diagnosis and pregnancy termination as influenced by the severity of the identified CFTR mutations.

CF screening is offered sequentially to couples in which at least one partner has any Caucasian ancestry. A panel of 37 CFTR mutations with reflex poly T testing is used. Acceptance of testing is high. Approximately 50,000 individuals (mostly women) have been screened and 1,776 carriers detected with an overall carrier identification rate of 1/28. Of the 37 mutations, 35 have been detected at least once. Data will be presented on 63 pregnancies in 50 high-risk couples. Interesting findings include the high incidence of several mild mutations, the role of modifying CFTR loci in determining phenotype, the ascertainment of asymptomatic compound heterozygotes, and the high volume of requests for repeat screening.

Since the inception of the screening program, the overall incidence of CF in our HMO population has been reduced by more than 50%. Therefore, the institution of prenatal CF screening has resulted in a dramatic redistribution of health care resources away from direct CF care towards CF prevention through avoidance. The projected cost-savings is favorable relative to the cost of medical care had CF screening not been available and provides additional rationale for prenatal CF screening from a policy perspective. The most obvious benefit is the knowledge a couple can acquire about the risk for the birth of a child with a severe, chronic, life-shortening disease and the option to avoid it or plan for it. This data will be updated at the presentation with an estimated additional 7,000 women screened.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 256 from 2003 ASHG Annual Meeting

Increased sensitivity at the cost of increased referrals when population-based newborn screening incorporates testing for multiple mutations (MM): Cystic fibrosis (CF) newborn screening (NBS) as a model.

A. Comeau[1], *R. Parad*[1,4], *H. Dorkin*[2,3], *M. Dovey*[4], *K. Haver*[3], *A. Lapey*[3], *R. Gerstle*[5], *B. O'Sullivan*[6]. 1) New England Newborn Screening Program, U Mass Med Sch; 2) New England Med Ctr; 3) Mass General Hosp; 4) The Childrens Hosp, Boston, MA; 5) Baystate Med Ctr, Springfield, MA; 6) U Mass Memorial Health Care, Worcester, MA.

With over 1000 mutations identified in the CFTR gene, numerous genotypes are observed in CF patients from various racial and ethnic groups. CF NBS strategies relying on elevated immunoreactive trypsinogen (IRT) and analysis for a single common CFTR mutation might miss some CF infants in genetically heterogeneous populations. NBS that incorporates multiple CFTR mutation (MM) testing and clinical observation may offer insights into increasing sensitivity and maximizing public health value. Feasibility and practical implications of CF NBS that includes MM testing were evaluated. A 30 month cohort of 202,721 infants born in MA between 2/1/99 & 8/1/01 were screened for CF at 2 days of age using a two tiered algorithm: all specimens were assayed for IRT; those with elevated IRT also had MM testing. Infants with positive CF screens were referred for sweat testing (ST).Outcomes included: Sensitivity, specificity, and predictive values between single and MM screening, relation to initial clinical presentation, and compliance with screening protocol. 73 of 75 CF affected infants (PV^N 99.99%) were detected. Among affected babies with elevated IRT, 90%; could have been detected with single mutation vs. 96% detected with MM testing. This increased sensitivity yielded 28% more carrier identifications and referrals. 26% of CF infants heterozygous for the common mutation revealed a second mutation with MM testing and were recognized earlier as affected. Detection of CF babies with "not common" CFTR mutations required a subset of babies with extremely elevated IRT to undergo ST regardless of CFTR mutation detection. Utilization of MM testing improved post screening prediction of CF at the cost of increased carrier identification and referrals.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 257 from 2003 ASHG Annual Meeting

Newborn screening blood samples: a vulnerable DNA database. *M. Lewis*[1], *L.L. McCabe*[1,2], *E.R.B. McCabe*[1,2]. 1) Dept Peds, David Geffen Sch of Med at UCLA and Mattel Children's Hosp at UCLA, LA, CA; 2) Dept Hum Gen, David Geffen Sch of Med at UCLA, LA, CA.

Critics of existing DNA databases caution that a time will come when a blood sample will be drawn from every newborn baby and stored for future use. That day has already arrived. State law requires every infant in most states to undergo newborn screening (NBS). Residual blood samples from these tests have a broad range of potential uses, from developing new genetic tests to analyzing epidemiological data. Residual samples also could serve as a DNA database. We evaluated the laws governing retention and use of residual blood samples in state NBS programs. State statutes form the foundations for regulations. State statutes regarding retention and use of NBS blood samples from all fifty states plus the District of Columbia were analyzed. Current legislation in six states addresses retention and use of NBS blood specimens. In one state, release of blood samples for confidential, anonymous study is authorized by statute. In two states, state departments of health may authorize use of NBS blood samples for research purposes. In two other states, state departments of health are directed to develop a schedule for retention and disposal of NBS blood samples. In both states, specimens may be used for medical research during the retention period, and parents are to be given educational materials that include an explanation that samples may be used for research purposes. Only one of these states requires written, informed consent from parents. Legislation in one state specifically prohibits the state department of health from requiring use of NBS samples for the purpose of obtaining complete genetic information. Paucity of legislation on use of retained NBS samples demonstrates the need for model regulatory language to govern retention and use of stored NBS blood specimens. This language should address how specimens should be stored, who should have access to the samples and for what purposes, transfer of the samples, and when informed consent from parents is required. DNA databases represented by NBS samples are largely unprotected by legislation and are therefore vulnerable to misuse.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 258 from 2003 ASHG Annual Meeting

Measuring the quality of informed consent (QuIC) of participants in a population-based genetic database. *A. Cirino, W. Wolf, R.L. Chisholm, I. Helenowski, K. Ormond.* Northwestern University, Chicago, IL.

Population-based genetic research studies are currently undertaken to better understand complex, common diseases. Despite a large body of literature on the importance of informed consent in genetic research, there is little quantitative research data on this topic. The goals of this research were to assess the knowledge and self-assessed understanding of participants in a population-based genetic study and to identify factors predicting participation and comprehension in such studies. Interviews were conducted with 135 individuals enrolled in the NUgene project (43.7%), a longitudinal study to associate medical information with genotype data at Northwestern University; data collection will continue to reach 200 subjects. Telephone interviews were completed within 8-64 days (mean=22.4 9.2) of NUgene enrollment and consisted of: demographic questions, a modified version of the quality of informed consent measure (QuIC), and semi-structured questions about participation and understanding of NUgene (data presented separately). Knowledge scores and self-assessment scores for the modified QuIC were generated (range 0-100). NUgene participants self-assessed understanding (89.4) was greater than their actual knowledge (68.6). Participants had a good understanding of the nature of the study (100), that the potential benefit is for future patients (99.6), that participation is voluntary (92.6), and a reasonable understanding that the research is not intended to benefit them (76.9). Less understood concepts included: potential risks and discomforts (16.7), the experimental nature of the genetic testing (22.2), procedures to follow in the event of injury (38.5), and confidentiality issues (42.2). In contrast to the therapeutic misconception reported in clinical research involving unproven treatments, NUgene participants report understanding that the purpose of the study is to benefit future patients and not expect personal benefit. However, participants reported a better understanding than knowledge scores indicated. Identifying common misconceptions can help to decrease this incongruity and improve informed consent for population-based genetic research. .

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 259 from 2003 ASHG Annual Meeting

Adding the Public's Voice to Policy Making Around Reproductive Genetic Technologies. *K. Hudson[1], B. Bernhardt[3], T. Doksum[2], D. Doukas[3], J. Evans[4], G. Geller[1], A. Kalfoglou[1], L. LeRoy[2], D. Mathews[1], N. Reame[5], J. Scott[1], The Reproductive Genetics Qualitative Research Team (in alphabetical order).* 1) Johns Hopkins Univ, Washington, DC; 2) Abt Associates, Inc. Cambridge, MA; 3) University of Pennslyvania, Philadelphia, PA; 4) University of California San Diego, San Diego, CA; 5) University of Michigan, Ann Arbor, MI.

The public's voice is frequently missing from debates about reproductive genetic technologies. To fill this gap, we collected data on public attitudes as part of an ongoing project to develop policy options to guide the development and use of these technologies. In the Fall of 2002, we conducted a random-digit dial, national survey of 1211 U.S. residents. Survey respondents' views differed significantly based on whether they approach these issues primarily in terms of health and safety or religion and morality. To explore the public's views and values shaping these views more fully, we conducted 21 focus groups in five U.S. locations in April 2003. Participants were assigned to groups based on age, sex, race, religion, education, and parental status, and were asked to comment on scenarios involving carrier testing, preimplantation genetic diagnosis, prenatal diagnosis, genetic modification, and sex selection. Transcripts were analyzed using the qualitative data analysis program NVIVO 2.0. Participants identified a range of health, psychological, economic, social, and global consequences of these technologies. Attitudes were shaped by participants' underlying values, including alleviating illness and suffering; preventing harm; improving quality of life; preserving the life of embryos/fetuses; ensuring safety and efficacy; avoiding discrimination, stigma, and inequality; and maintaining human diversity. Participants engaged in robust, nuanced discussion about whether technologies ought to be used in particular circumstances and who should make these decisions. Discussions did not mirror the traditional pro choice/pro life debate, but rather reflected a deep commitment to individual/family autonomy. We will report research findings and describe how these data will be used to design policy options to guide the use of these technologies.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 260 from 2003 ASHG Annual Meeting

Decision making process in the context of genetic risk. *L. Gerad*[1], *S. Shiloh*[2], *G. Barkai*[1], *M. Berkenstadt*[1], *M. Frydman*[1], *B. Goldman*[1]. 1) Insititure of human Genetics, The Chaim Sheba Medical Center, Ramat Gan 52621, Israel; 2) Department of Psychology, Tel Aviv University, Ramat Aviv 69978, Israel.

This study explored decision-making processes of genetic counselees within the theoretical framework of psychological theories of decision-making under risk.Methods:161 genetic counselees facing reproductive decisions participated in the study. A semi-structured AIS (Active Information Search) interview was conducted before counseling. This allowed following counselees' decision process through their information search. Questions asked by counselees during counseling sessions served as another source of data. Counselees' questions were analyzed using a two-dimensional coding system:1) information content (diagnosis, etiology, consequences, control of negative outcomes),2) level of certainty requested (precise probability, imprecise probability, ambiguity).In addition, Individual differences in perceived personal control, information search and health locus of control were assessed by standard questionnaires, and their effects on information search were investigated. Results: A selective information search pattern was found: consequences of the medical problem appeared as the most important information requested by counselees, followed by information about means for controling possible negative outcomes. Information about etiology and precise diagnosis played a minor role in the process. The majority of counselees wanted to know the exact probability for a negative outcome. Information search was negatively correlated with perceived control, and positively correlated with individual differences in medical information seeking. Significant differences in type of information requested were found between sub-groups of counselees considering different decisions as prenatal diagnosis, pregnancy termination, conception. Conclusions: Genetic counselees' decision process seems to include two major phases: cognitive appraisal of the health threat and cognitive appraisal of control. This may reflect a rational process of risk-management rather than risk decision-making. Individual and decisional differences seem to moderate information-seeking within the decision process.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 261 from 2003 ASHG Annual Meeting

The impact of genetics on individuals and society: Voices of Black Americans. *C.B. Denton[1], T.E. Jayaratne[2], J.P. Sheldon[3], A.R. DiNardo[1], M.B. Feldbaum[2], E.M. Petty[1]*. 1) Int Medicine & Human Genetics, Univ of Michigan, Ann Arbor, MI; 2) Institute for Research on Women & Gender, Univ of Michigan, Ann Arbor, MI; 3) Dept of Behavioral Sciences, Univ of Michigan-Dearborn, Dearborn, MI.

As studies of human genetic variation increase, serious consideration of the potential impact on members of our society is critical. Some fear identification of genetic differences between ethnically or racially defined groups could lead to injurious societal outcomes. Others believe that such information would be beneficial, especially in health care settings. Given our history of eugenics and racism, perspectives of Black Americans are particularly important to consider. As part of an IRB-approved NIH-ELSI study we conducted open-ended telephone interviews with 43 Black individuals chosen out of 1200 randomly selected adults from our earlier structured closed-ended survey, examining how people use genetics to support their underlying beliefs about individual and group differences as related to human traits. Individuals were asked whether they felt increased genetic knowledge might be helpful or harmful. A qualitative analysis coding tool categorically organized the transcripts by thematic content.

Only 25% of respondents believed genetics played a significant role in individual differences in athletics, violent tendencies, and intelligence. While most Blacks surveyed felt that genes were not involved, over two-thirds believed Whites would say that differences in these traits were genetic. Of these, 25% felt that White people thought they were genetically superior. Close to 50% of respondents believed genetic advances could help Black people, although over 50% felt that genetics could harm the Black population. Around 30% believed it could increase racism and stereotypes. Over 10% felt Blacks could be used as lab rats in genetic experiments. Soliciting these and additional opinions from minority groups is essential to understanding their underlying genetic beliefs and related concerns. Only through awareness and appreciation of such concerns can we translate genetic advances into resources that maximize benefits and minimize misuses for all members of our society.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 262 from 2003 ASHG Annual Meeting

What people of diverse cultures think about genetics: an urban experience towards community-based genetic education. *F. Wolman, MD, MPH[1], D. Catz, PhD, MPH[2], J.N. Tobin, PhD[2], M. Puryear, MD, PhD[3], P. Kyler[3], N. Green, MD[1], A. Umemoto[1], J. Cernoch, PhD[4], R. Brown[5]*. 1) March of Dimes, White Plains, NY; 2) Clinical Directors Network, Inc., NY; 3) Genetic Services Branch, MCHB, HRSA, Rockville, MD; 4) Family Voices, Albuquerque, NM; 5) Genetic Alliance, Washington DC.

Purpose: The goal of the HRSA-funded Genetic Education and Needs Evaluation (GENE) project is to develop community-based, culturally appropriate strategies to improve minority access to genetic information.

Methods: Five focus groups were conducted in English, two in Spanish and one in Mandarin to understand attitudes and beliefs towards genetics among different cultures. Participants included 27% African Americans, 36% Hispanics, 22% Caucasian and 15% Asian (Chinese).

Results: Participants knowledge of genetics, genetic testing and newborn screening was limited. Minority participants main source of health information came from pamphlets or booklets, the media and professional training. In contrast, white participants looked for health information from journals, the Internet and the media. Most participants indicated that they needed more information about genetics in general, and about genetic testing and newborn screening. To participants, the possibility of preventing diseases was the major advantage of genetic testing. The main concerns were: anxiety, insurance restrictions, financial burden, privacy and racial/ethnic discrimination. Most of the participants would agree to have carrier testing and newborn screening, but only half of them would be willing to undergo genetic testing without reservation.

Conclusions: Regardless of cultural background, participants had almost the same expectations and concerns and agreed on the need for genetic education. With the new genetic tools being developed, it will be necessary to provide culturally- and literacy- appropriate genetic education to ensure that underserved populations can make informed choices about their health.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Using telemedicine to provide clinical genetics services and genetics education in Maine; Results of three year pilot trial. *R. Smith*[1], *D. Lea*[1], *S. Ellingwood*[1], *S. Schulberger*[2]. 1) Southern Maine Genetics Services, Foundation Blood Research, Scarborough, ME; 2) Maine Chapter, March of Dimes.

As of October 2003, Southern Maine Genetics Services at FBR will complete a 3 year pilot trial studying the viability of using telemedicine as a delivery tool to provide clinical genetic and educational services to underserved areas of Maine. These services are now provided to 25 sites throughout the state. There were 17 consults in year one, increasing to 45 in year two, with a predicted 50 in year three based on current numbers. The increase in referral numbers was due in part to increasing enrollment of telemedicine sites, as well as education of providers regarding appropriate referrals. Referral questions included a variety of pediatric and adult concerns. Educational sessions were specifically tailored for small, targeted, interdisciplinary audiences, numbering 13 in year one, 37 in year two, and 36 by June 2003. Provider and patient survey results of this project were obtained. Positive perceptions from providers included; patients staying under providers care, choice of attending consult, and the ability to discuss recommendations immediately with the genetics team. Patients noted avoidance of travel and travel costs as a major advantage. Negative perceptions by providers included nonfamiliarity with the technology and the feeling that genetics services were not of major importance to their patient's care. Some patients would have preferred face to face contact. From our standpoint, telemedicine allowed us to counsel and evaluate patients who in many instances would have not otherwise sought the service. We found that rapport could satisfactorily be established during a counseling session. The technology allowed for adequate dysmorphology exam, but was somewhat dependent on patient cooperation and appropriate training of on site assistant. Project sustainablily is now our prime focus through obtaining insurance reimbursement and additional grant support. Overall, we feel the project has been successful in piloting telemedicine as a viable alternative delivery tool for genetic services to Maine's rural population and establishes a model for other states to follow.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 264 from 2003 ASHG Annual Meeting

Psychological impact of genetic counselling for familial cancer: a systematic review and meta-analysis. *J. Emery, D. Braithwaite, F. Walter, T. Prevost, S. Sutton.* Public Health & Primary Care, University of Cambridge,UK.

Aim: To systematically review the evidence relating to psychological outcomes of genetic counselling for familial cancer.

Methods:6 electronic databases were searched to identify controlled trials or prospective studies that examined the effect of genetic counselling on risk perception, knowledge, anxiety, cancer-specific worry, depression and cancer surveillance. 42 papers were reviewed in detail and 25 met inclusion criteria. 22 related to breast cancer, 2 to colorectal and 1 to ovarian cancer. 7 papers reported on 4 randomised controlled trials and 1 controlled trial. Data were extracted according to pre-specified criteria. Effect sizes were computed for both short-term (1 month) and long-term (3 months) follow-up. For the controlled trials, effect sizes were pooled using random-effects meta-analyses.

Results: In controlled trials genetic counselling improved knowledge of cancer genetics (pooled short-term effect +0.70,95%CI +0.15 to +1.26) but did not alter the level of perceived risk (pooled short-term effect -0.10, 95% CI -0.24 to +0.04). Prospective studies reported improvements in the accuracy of perceived risk (p=0.016). No effect was observed on general anxiety (pooled long-term effect +0.05, 95%CI -0.21 to +0.31) or cancer-specific worry (pooled long-term effect 0.15, 95%CI -0.35 to +0.06) in controlled trials. In contrast, prospective studies demonstrated short-term reductions in general and cancer specific anxiety. Few studies examined cancer surveillance behaviours and no studies attempted to measure informed decision making.

Conclusions: Genetic counselling for familial cancer leads to improvements in knowledge about cancer genetics without any adverse psychological effects. Differences in findings between controlled trials and prospective studies reflect the problem of defining suitable comparison groups in trials of genetic counselling. Future trials of genetic counselling should consider carefully the choice of most appropriate comparison group and measure informed decision making and cancer surveillance behaviours.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 265 from 2003 ASHG Annual Meeting

Recombination Rates, their local variation and the estimated number of hotspots in the human genome. *M.P.H. Stumpf*[1], *G.A.T. McVean*[2], *S. Myers*[2], *E. DeSilva*[1]. 1) Department of Biology, University College London, London, UK; 2) Department of Statistics, University of Oxford, Oxford, UK.

We estimate recombination rates, their local variation and the density of recombination hotspots in the human genome. Two estimators, a composite likelihood approach that uses genotypic data without having to infer haplotypes, and a non-parametric estimate for the minimum number of local recombination events are applied to the data of Gabriel et al. (Science, 296 (2002) 2225-2229). We find considerable variation in the local recombination rate between and within the different regions but high levels of concordance for estimates obtained from different populations (correlation of recombinatioin rates between populations: 0.85-0.95). There is also excellent agreement between the different estimators. Estimated recombination rates appear to give a much more consistent description of the linkage disequilibrium (LD) structure than do the simple summary statistics is use today. We also provide evidence that demographic uncertainty introduces no or only little bias into our estimators. A detailed study of local recombination rate variation detects several features that can be described as recombination hotspots. Comparison with the data of Jeffreys et al. (Nat.Gen., 29 (2001), 217-222) allows us to estimate the number of recombination hotspots that can be detected at the average marker density (approximately 1/5kb) in the data. Our conservative estimate yields approximately 15 000-20 000 hotspots/hotspot clusters in the human genome. We show that this number is likely to go up as denser marker sets are considered.An extensive simulation study confirms the statistical power of the estimators and the low levels of bias resulting from different demographic histories and marker density/choice. Both estimators of the recombination rate are computationally inexpensive and considerably aid in the interpretation of LD or haplotype diversity data.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Measures of similarity for haplotype block structures show variation among populations. *N. Liu*[1], *S. Sawyer*[2], *N. Mukherjee*[3], *A.J. Pakstis*[3], *J.R. Kidd*[3], *K.K. Kidd*[3], *A. Brookes*[2], *H. Zhao*[1,3]. 1) Dept Epidemiology and Public Health, Yale U., New Haven, CT; 2) Karolinska Institute, Stockholm; 3) Dept Genetics, Yale U. School of Medicine, New Haven, CT.

Several methods have been proposed to identify haplotype blocks and haplotype tagging SNPs. However, many aspects of the block-like structures and tag SNPs are poorly understood. For example, little is known about similarity of block structures and tag SNP sets across worldwide populations, a question with significant impact on the construction of haplotype block maps that may be useful across all ethnic groups. In this presentation, we report a systematic study to characterize haplotype structure similarities across many worldwide populations. To facilitate such comparisons, we first propose several similarity measures for objectively comparing haplotype block partitions and tag SNP sets. These similarity measures are analytic and equipped with statistic tests. Our haplotype block partition similarity measures take into account the block boundary position information and are more accurate than existing methods. No similarity measure for tag SNP sets has previously been proposed. We have analyzed a SNP dataset of 1830 chromosomes drawn from 16 worldwide populations. Our results show (1) that block boundaries can vary among populations with European populations and some African populations showing similar boundaries but other populations showing other patterns of block boundaries. Block structure is occasionally very similar for some populations from very different geographic regions. (2) Tagging SNP sets are generally similar for populations with similar haplotype block structures but differ if the block structures differ. (3) All but one of the block finding methods we have tested yield generally consistent results although there exist variations. The concept of haplotype blocks is relatively robust for the various block measures. Our results show that it is unlikely that a common haplotype pattern exists for all human populations: many populations, even in the same geographical region, may have different haplotype patterns. Our findings have clear implications for mapping complex disease genes.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 267 from 2003 ASHG Annual Meeting

Distribution, Sharing, and Ancestry of Common Haplotypes in African-American, Caucasian, and Asian Populations. *F.M. De La Vega, H. Avi-Itzhak, B. Halldórsson, C. Scafe, S. Istrail, D.A. Gilbert, E.G. Spier.* Applied Biosystems, Foster City, CA.

The extent and patterns of statistical association between neighboring alleles of sequence variants (linkage disequilibrium or LD) is the basis of all genetic mapping and the result of the interplay between recombination, natural selection, genetic drift, and population history. We performed a comparative study of the LD profile across three human autosomes: chromosomes 6, 21, and 22. Over 20,000 SNPs with a median spacing ranging from 4 to 7 kb were genotyped in African-American, Caucasian, Chinese, and Japanese population samples. We identified haplotype blocks, regions of strong LD and low haplotype diversity, by a rule-based method based on evidence of recombination optimized to minimize the total number of blocks across a chromosome. By examining the segments where haplotype blocks overlap between two populations, we found that 70% of the common haplotypes are shared between African-Americans and Caucasians, consistently across the three chromosomes. Over 23% of the most common haplotypes in these segments are only found in African-Americans, whereas only 5.8% are exclusively found in Caucasians, implying that greater haplotype diversity exists in the African-American population. We were able to infer the ancestral haplotype for a number of block overlaps by genotyping chimpanzee DNA samples. In about 13% of the segments analyzed, the ancestral haplotype was exclusively present in the African-American blocks, whereas the ancestral haplotype was represented solely in Caucasiansin only 1.2% of them. Moreover, when the ancestral haplotypes were found in both populations, their frequencies tended to be higher in the African-American population. Similar analyses of the Asian population samples are also presented. The haplotype patterns observed are compatible with the existence of a past bottleneck in the human population that migrated from Africa to inhabit Europe and Asia, the latter two populations showing significantly more extensive LD.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 268 from 2003 ASHG Annual Meeting

Defining haplotype blocks in the human genome: The impact of methods, parameters and allele frequencies. *T.G. Schulze*[1, 2,3], *K. Zhang*[4], *Y.-S. Chen*[3], *N. Akula*[2,3], *F. Sun*[4], *F.J. McMahon*[2]. 1) Division of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Mannheim, Germany; 2) Mood and Anxiety Disorders Program, National Institute of Mental Health, National Institutes of Health, Bethesda, MD; 3) Department of Psychiatry, The University of Chicago, Chicago, IL; 4) Department of Biological Sciences and Mathematics, University of Southern California, Los Angeles, CA.

It has been proposed that much of human genome is organized in blocks of haplotypes, but there is as yet no consensus about the best way to define haplotype blocks. We compared two major methods for defining haplotype blocks, the LD method (Gabriel et al. 2002) and the Dynamic Programming Algorithm (DPA) (Zhang et al. 2002). To increase the generalizabiliy of our study, we studied 3 chromosomal regions. The first region is a 180 kb contig of finished sequence on 18q21.32-33, genotyped on unrelated European CEPH founders. The second and third regions are located on chromosome 22 (publicly-available data from unrelated Europeans /Wellcome Trust Sanger Institute): 22q13.31-32 (812 kb) and 22q13.33 (993 kb). For each analysis, we varied the minor allele frequency (q) from 1% to 41 %. Each method produced strikingly different results. DPA consitently identified fewer and larger haplotype blocks and fewer tag SNPs than the LD method. For both methods, the results critically depended on q. Decreasing q produced an up to 3.7-fold increase in the number of haplotype blocks and tag SNPs. Block definition was also heavily influenced by the choice of parameter values. Our results demonstrate that the detection of haplotype blocks depends on the algorithm, the choice of parameter values, and, most importantly, the marker allele frequencies. It is widely hoped that a haplotype map will make genome-wide association mapping economically and statistically feasible. If valid haplotype blocks exist, then appropriate statistical methods should provide convergent results. Our findings show that 2 widely-used methods do not provide convergent results. The detection of valid haplotype blocks useful for association mapping may be more difficult than previously believed.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 269 from 2003 ASHG Annual Meeting

MSR1 **haplotype variation and prostate cancer risk in populations of African descent.** *L.O. Long[1], L. Zheng[2], T. Lewis-Smith[1], C.A. Ahaghotu[1], A. Jackson[1], V. Freeman[3], C. Ewing[4], J. Xu[2], W. Isaacs[4], R.A.. Kittles[1].* 1) National Human Genomce Center, Howard University, Washington, DC; 2) Wake Forest University School of Medicine, Winston-Salem, NC; 3) Loyola University, Chicago, IL; 4) Dept. of Urology, Johns Hopkins University, Baltimore, MD.

The macrophage scavenger receptor 1 (MSR1) functions in the innate immune defense and inflammatory response mediated by macrophages. Analysis of hereditary prostate cancer (HPC) patients revealed several rare mutations of the *MSR1* gene linked to prostate cancer. Recently, several sequence variants and haplotypes common among European Americans were associated with prostate cancer risk in non-HPC patients. To elucidate the significance of these novel sequence variants in prostate cancer risk, we genotyped the five common sequence variants (PRO3, INDEL1, IVS5-59, P275A, and INDEL7) in non-HPC African American (N= 289) and Jamaican (N=303) prostate cancer cases and healthy controls. Genotyping was performed using MassArray (SEQUENOM) and Pyrosequencing. MSR1 haplotypes were estimated from the five marker genotypes by the EM algorithm. Allele frequencies were quite distinctive between European American and African descent control populations for the PRO3 G and INDEL + alleles. Patterns of linkage disequilibrium were not conserved among the African descent populations when compared to European Americans. Contrary to what was observed among European Americans, only three of the five SNPs (INDEL 1, IVS5-59, and P275A) were associated with prostate cancer risk in the population of African descent. A completely different haplotype, A(-)CG(-), conferred increased risk for prostate cancer in the African American (OR=4.4; 95%CI=1.2-16.7) and Jamaican (OR=4.7; 95%CI=1.0-62.8) populations. Functional studies of the SNPs reveal that the mutant P275A allele exhibits a ~30% decrease in ligand binding activity. This study provides strong evidence that the common *MSR1* P275A allele likely contributes to increased risk for sporadic prostate cancer. In addition this study demonstrates the utility of using populations of African descent for haplotype and fine mapping studies.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Haplotype diversity across 100 candidate genes for inflammation and lipid metabolism. *D.C. Crawford[1], C.S. Carlson[1], M.J. Rieder[1], M.A. Eberle[2], L. Kruglyak[2], D.A. Nickerson[1].* 1) Dept Genome Sciences, University Washington, Seattle, WA; 2) Fred Hutchison Cancer Research Center, Seattle, WA.

Recent studies have suggested that the genome consists of high linkage disequilibrium blocks ranging from 5 to >100kb in length. Within these blocks, a few haplotypes (3-5) may explain >90% of the observed haplotypes. These studies also suggest that common haplotypes are shared across populations and represent ~80% of the chromosomes. These observations, however, are based on an incomplete knowledge of the genetic variation in the regions examined. To bridge this gap in knowledge, we at SeattleSNPs have completely re-sequenced 100 candidate genes involved in inflammation and lipid metabolism in two populations: African-descent (n=24; AD) and European-descent (n=23; ED). While well studied as genes of medical importance, we demonstrate that there is more genetic variation within these genes than previously documented. We identified 8,877 biallelic polymorphisms, resulting in an average density of 5.37 per kb or 77.93 (AD) and 46.20 (ED) per gene. Using common sites (minor allele frequency >5%) to infer haplotypes using PHASE, we also demonstrate that the average number of haplotypes per gene is higher than studies of coding region variation suggest: 22.65 (AD) and 13.25 (ED). This finding extends to common haplotypes as well. While the average number of haplotypes with a frequency >5% is ~5 for both populations, the AD population has a range of 0 to 11 common haplotypes compared with the range of 1 to 8 in the ED population, demonstrating gene to gene variation across the two populations. Furthermore, we demonstrate that haplotypes shared between the two populations only constitute a fraction of the total number of haplotypes observed (17.9%), and these shared haplotypes represent only 42% and 70% of AD and ED chromosomes, respectively. Finally, we demonstrate that choosing common sites every 5 kb and inferring haplotypes captures only 23% (AD) and 31% (ED) of the average number of haplotypes that can be observed. These data suggest that the genome is more complex than previously described, which may have an impact on future genome-wide and candidate gene association studies.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 271 from 2003 ASHG Annual Meeting

Towards a fine-scale linkage disequilibrium map of human chromosome 20. *L.R. Cardon[1], X. Ke[1], R. Lawrence[1], N. Carter[2], J. Rogers[2], G. Stavrides[2], D. Willey[2], J. Mullikin[2], S. Hunt[2], D.R. Bentley[2], P. Deloukas[2].* 1) Wellcome Trust Ctr Human Genet, Univ Oxford, Oxford, United Kingdom; 2) Wellcome Trust Sanger Institute, Hinxton, United Kingdom.

Genome-wide maps of linkage disequilibrium are being developed to facilitate association studies of complex diseases. At present, it is not clear how many SNPs will be needed to meet this objective, nor how to usefully assess LD to guide marker selection. Various definitions of haplotype blocks have been put forth, as have different metrics based on genetic maps. Until recently, little genomic data were available to fruitfully compare such measures. We have constructed a dense map < 1 SNP/2 kb of chromosome 20 to evaluate LD profiles and statistical measures. Chromosome 20 was flow-sorted from a Caucasian, an African American, an African Pygmy and a Chinese cell line, and individual small-insert libraries were shotgun sequenced to a depth of 2x coverage. Over 130,000 new SNPs were discovered, of which 60,000 assays have been designed and are being genotyped across DNA panels of CEPH families, and unrelated Caucasians, African-Americans and Asians. We have completed a 10 Mb region of 20q12-13.2, comprising 5293 SNPs with minor allele frequency >= .04. To evaluate the effects of map density and allele frequency, we took random subsets of the dense map and applied the most commonly used block definitions and genetic map-based methods, then assessed the robustness of the different approaches against the unselected marker panel. The results indicate the extent to which LD at coarse SNP densities reflects genuine patterns and illustrate the strengths/weaknesses of the different statistical approaches. We show that sequence coverage and boundaries of haplotype blocks are dependent on marker density, such that increasing density yields more blocks of apparently shorter length. Genetic-map based approaches appear more robust to marker density, but can be heavily influenced by the allele frequencies of the markers selected to study. These outcomes have implications for association studies, which we discuss in the context of future expectations and past examples.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 272 from 2003 ASHG Annual Meeting

The first metric linkage disequilibrium map of a human chromosome. *W.J. Tapper, N. Maniatis, N.E. Morton, A. Collins.* Human Genetics, School of Medicine, General Hospital, University of Southampton, Southampton, SO16 6YD, UK.

Recent descriptions of linkage disequilibrium (LD) patterns have focused on delimiting blocks corresponding to regions of low haplotype diversity. The HapMap project intends to aid positional cloning by determining common haplotype patterns within these blocks so that only a few haplotype tag single-nucleotide polymorphisms (htSNPs) need to be typed to define each haplotype. However, there are difficulties since block definitions are arbitrary and only a proportion of the genome is composed of blocks. Furthermore, the relationship between blocks is important since LD may extend across blocks. In comparison, a metric LD map, with map distances analogous to the centiMorgan (cM) scale of linkage maps provides additional information by characterising inter-block regions which define the relationship between blocks. Such maps avoid arbitrary block definitions and give an additive scale that is useful to determine optimal marker spacing for positional cloning. LD maps also provide insights into the relationship between sequence motifs and recombination since the pattern of LD is closely related to recombination hot-spots and their resolution is higher than existing linkage maps. Using LDMAP to analyse SNP data spanning chromosome 22 (Dawson et al., 2002), we have obtained the first whole-chromosome metric LD map. This map identifies regions of high LD as plateaus and regions of low LD as steps reflecting variable recombination intensity. The intensity of recombination is related to the height of the step and thus intense recombination hot-spots can be distinguished from more randomly distributed historical events. The map identifies holes in which greater marker density is required and defines the optimal SNP spacing for positional cloning which suggests that some multiple of around 50,000 SNPs will be required to efficiently screen Caucasian genomes. Further analyses which investigate selection of informative SNPs and the effect of SNP allele frequency and marker density will refine this estimate. The map is also closely correlated with the most recent high-resolution linkage map and a range of sequence motifs including GT/CA repeats and GC content.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 273 from 2003 ASHG Annual Meeting

How useful are the tagging SNPs for identifying complex disease genes? *H. Zhao[1,2], R. Pfeiffer[2], M. Gail[2]*. 1) Epidemiology & Public Health, Yale Univ Sch Medicine, New Haven, CT; 2) 2Biostatistics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD.

Although millions of genetic polymorphisms have been identified in the human genome, due to linkage disequilibrium, a small proportion of these markers may suffice tobe needed to capture the majority of the diversity due to linkage disequilibrium and to identify complex disease genes. Several methods have been proposed to select these representative markers, commonly called haplotype tagging SNPs. One use of these representative markers is to study aspects of population genetics. For this purpose, selecting markers that capture the diversity or otherwise represent the full set of markers seems reasonable, and selection can be based on a random sample of individuals from the population. A second goal is to select a set of markers to detect an association of disease with haplotype (disease gene mapping). We investigated the usefulness of three methods that have been proposed to select markers that preserve information or diversity in population based samples for identifying disease associations with haplotypes in case-control studies:. entropy, a haplotype diversity statistic defined by Clayton, and Strams correlation measure. We examined five genes (ADH, APOE, CCR2/CCR5, COMT, NOD2) with known disease associations. We found that these three selection criteria procedures designed to preserve information or diversity often lead to the selection of markers with poor power to detect the disease association. In contrast to these population-sample based tagging SNP selection criteria, Aalternative strategies that incorporate disease information in SNP selections, such as a two-stage design that uses data on cases and controls in the initial stage, may offer a more powerful approach to selecting most informative markers for disease gene identification.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 274 from 2003 ASHG Annual Meeting

Determinants of success in genetic association studies of complex traits. *K. Zondervan, A. Morris, L. Cardon.* Wellcome Trust Ctr Human Gen, Univ Oxford, Oxford, United Kingdom.

Case-control studies of multifactorial diseases and genetic variants have been notable by their lack of success and replicability, partly due to poor epidemiological practice. However, the apparent effect size - the marker odds ratio (OR) - is statistically determined by 4 parameters: the OR of the disease variant; disease and marker allele frequencies (DAF & MAF), and linkage disequilibrium (LD) between marker and disease variant. We derived this relationship, showing that under complete LD, and MAF>DAF, the relationship between marker and disease OR reduces to a simple expression. We used 5 complex disease associations to illustrate: Deep Vein Thrombosis & FVL (DAF: 0.03); Crohn's disease & NOD2 (0.06); Alzheimer's & APOE (0.15); Bladder cancer & GSTM1 (0.7); and NIDDM & PPAR (0.85). Empirical LD data from chromosome 19 was used to investigate the variability in common MAFs within haplotype blocks.

ORs of the low frequency disease alleles were substantial: 3.3-4.6 (allelic ORs), and 11.7-40.0 (homozygote GRRs). Using a range of marker frequencies in moderate LD with the disease loci (D'>0.5-0.6), allelic ORs decreased considerably but remained detectable with 80% power in a sample of 1000 cases and 1000 controls. ORs for the common disease alleles were low (allelic ORs: 1.2-1.3; GRRs: 1.5-2.0). Using samples of 5000 cases and 5000 controls, MAFs had to closely resemble DAFs to allow detection with 80% power but only when D'>~0.7. Chromosome 19 data showed that ~60% of markers were within 0.1 of the most common marker frequency within blocks.

Associations of complex traits with rare alleles conferring large ORs, and common alleles with modest ORs should be detectable in large case-control studies using common markers (MAF > 0.1) and information on genomic LD patterns. Currently, rare alleles with small ORs will not be detectable in feasible samples, unless rare markers in very high LD with the disease locus are used. The development of the HapMap aimed at providing a genomic map of LD may provide help in study design.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Recombination rate and reproductive success in humans. *A. Kong[1], J. Barnard[1], D.F. Gudbjartsson[1], G. Thorleifsson[1], G. Jonsdottir[1], S. Sigurdardottir[1], B. Richardsson[1], J. Palsdottir[1], T. Thorgeirsson[1], M.L. Frigge[1], N. Lamb[2], S. Sherman[2], J.R. Gulcher[1], K. Stefansson[1].* 1) Decode Genetics, Reykjavik, Iceland; 2) Emory University, Atlanta, GA.

Using our Icelandic genealogy database, together with a recently compiled data set on recombinations, we study the relationships between recombination rates, parental age and family size. Based on genome-wide microsatellite marker data on 23,066 individuals, consisting of a total of 18.5 million genotypes, providing information on the recombination events of 14,140 maternal and paternal meioses each, we detected a modest but highly significant positive correlation between maternal recombination counts of an offspring and maternal age. Previous attempts to convincingly demonstrate such an effect have been hampered by their limited sample sizes. Interpretation of this observation should take into consideration that our data are based on successful gametes that yield live births. One possible explanation is that the recombination rate of eggs does not actually increase with maternal age, but is the consequence of selection. Specifically, we propose that a high recombination count increases the chance of a gamete to become a liveborn, and this effect becomes more pronounced with advancing maternal age. This interpretation is supported by existing knowledge on maternal nondisjunction. We provide further support by demonstrating that mothers with high recombination rates tend to have more children than mothers with low recombination rates. Thus, our data suggest that the reproductive success of an oocyte depends, in part, on the genome-wide recombination count. Not only do recombinations generate diversity of gene combinations to fuel natural selection, they are themselves subject to selection.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Gender, not genotype, determines recombination levels in mice. *A. Lynn, S. Schrump, J. Cherry, P. Hunt, T. Hassold.* Department of Genetics, Case Western Reserve University, Cleveland, OH.

The central feature of meiosis in diploid organisms is meiotic recombination, the precise physical breakage and rejoining of homologous chromosomes. Recombination rates vary, depending on physical size of the chromosome, chromosomal location, and proximity to other recombination events. Additionally, for reasons that are not understood, recombination varies with gender; e.g., in both mice and humans, females have higher levels of recombination than males. We hypothesized that this difference might be strictly genetically determined, with XY cells "destined" to have a lower level of recombination than XX cells. To test this hypothesis, we generated sex-reversed XY female mice, and compared the rates and patterns of recombination in the sex-reversed females to normal sibling XX females and XY males. Recombination rates were assayed directly in meiotic cells by immunostaining methods, using antibodies against MLH1 to mark the sites of cross-overs.

To our surprise, the overall rate of autosomal recombination in the sex-reversed XY females (26.7 3.5) was similar to rates observed in XX females (26.1 3.8), and markedly different than those of XY males (24.6 2.6). Further, among the sex-reversed XY females, the proportion of chromosomes with 0, 1, 2, or 3 cross-overs fit the female, but not the male, pattern. Finally, the placement of cross-overs along chromosomes also fit the female pattern: e.g., for chromosomes with a single cross-over, the distributions for both normal XX and sex-reversed XY females showed a normal bell-shape, while the XY males distributions were skewed, with a tendency toward distal cross-overs. Thus, simple genetic explanations (i.e., the presence of a Y chromosome) cannot account for gender-specific rates and patterns of meiotic recombination. Accordingly, we are now examining specific epigenetic processes and environmental factors to assess their role in generating these gender-specific differences.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 277 from 2003 ASHG Annual Meeting

Methylation reprogramming and chromosomal aneuploidy in cloned rabbit embryos. *T. Haaf*[1], *F. Dirim*[1], *M. Stojkovic*[2], *V. Zakhartchenko*[2], *E. Wolf*[2], *W. Shi*[2]. 1) Inst Human Genetics, Mainz Univ Sch Medicine, Mainz, Germany; 2) Inst Mol Animal Breeding, Gene Center, LMU Munich, Germany.

In the early diploid mammalian embryo, the paternal and maternal genomes undergo epigenetic reprogramming of the two very different gamete nuclei for somatic development and formation of totipotent embryonal cells. Active zygotic demethylation of the paternal genome but not of the maternal genome appears to be evolutionarily conserved in mammalian species, with the possible exception of sheep. Normal development of cloned embryos depends not only on proper methylation reprogramming of a somatic cell nucleus that has been introduced into an oocyte by nuclear transfer but also on normal ploidy of the cells. Species differences in preimplantation events could explain the variable but generally low cloning efficiency in sheep, cow, pig, mouse and rabbit. We have analyzed the global methylation patterns and ploidy of rabbit embryos, a species which is extremely difficult to clone. Anti-5-methylcytosine immunofluorescence staining showed that the equally high methylation levels of the paternal and maternal genomes are largely maintained from the zygote up to the 16-cell stage in both normal and cloned rabbit embryos. However, there was greater variation among individual cells in nuclear transfer embryos. Evidently, the nature of the reprogramming process is not the same in all mammalian species. Three-color FISH with chromosome-specific BACs demonstrated postzygotic non-disjunction in the vast majority of cloned two- to 32-cell embryos. We propose that disturbed cell cycle control and chromosomal aneuploidy are one important reason why mammalian cloning often fails.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Dynamic Histone Modifications of the X and Y chromosomes during their Transient Inactivation in Mammalian Spermatogenesis. *A.M. Khalil, F.Z. Boyar, D.J. Driscoll.* Pediatrics Genetics and Molecular Genetics & Microbiology, Univ. of Florida, Gainesville.

The mechanism for the transient X and Y inactivation during mammalian spermatogenesis is unknown. In contrast to X inactivation (Xi) in female somatic cells there is no DNA methylation in the promoter regions of housekeeping genes on the inactive X during spermatogenesis. Moreover, male mice carrying an ablated *Xist* gene demonstrate that Xi during spermatogenesis is *Xist/Tsix* independent. In order to explore other mechanisms that may account for the transient X and Y inactivation during spermatogenesis we examined histone modifications in mouse germ cells. By combining immunocytochemistry and FISH we found that the X and Y become underacetylated at histone 4 lysine 12 and lysine 16 at the pachytene stage. The underacetylation of X and Y at histone 4 lysine 12 persists until the end of meiosis while the X and Y become reacetylated at histone 4 lysine 16 by the diplotene stage. Unexpectedly, the X and Y are hyperacetylated at histone 4 lysine 5 and lysine 8 at the pachytene stage, and become underacetylated by the diplotene stage. The X and Y remain underacetylated at histone 4 lysine 5 until the end of meiosis; however, they become reacetylated at histone 4 lysine 8 by metaphase II. Moreover, there is striking hyperacetylation of X and Y at histone 3 lysine 9 in early to mid pachytene which diminishes by late pachytene and is replaced with histone 3 lysine 9 hypermethylation. This hypermethylation at histone 3 lysine 9 persists until the end of meiosis. Intriguingly, the X and Y are hypermethylated at histone 3 dimethyl lysine 4 and simultaneously undermethylated at histone 3 trimethyl lysine 4 throughout spermatogenesis. These novel findings are exciting since they are likely to: 1) account for the transient X and Y inactivation during male meiosis; 2) provide a window for a better understanding of the early histone modifications involved in the initiation of X inactivation in female somatic cells and genomic imprinting in gametogenesis; 3) reveal a novel system for deciphering the histone code and 4) provide valuable insights into mechanisms of gene regulation in general.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 279 from 2003 ASHG Annual Meeting

Homologous pairing of 15q11-13 imprinted domains in brain is developmentally regulated but deficient in Rett and autism samples. *K.N. Thatcher, D. Braunschweig, R.C. Samaco, J.M. LaSalle.* Med Micro & Imm, UC Davis Sch Med, Davis, CA.

Mutations in *MECP2* cause Rett syndrome (RTT), but have also been found in a few cases of Angelman syndrome (AS) and autism. Although MeCP2 is predicted to be a transcriptional repressor of methylated genes, *MECP2* mutations in RTT do not result in the dysregulation of several maternally imprinted genes from the AS/PWS region on 15q11-13. As elevated MeCP2 expression is acquired with increasing age in neurons, we hypothesized that MeCP2 may play a role in chromosomal organization during postnatal neuronal maturation. Homologous pairing of 15q11-13 alleles has previously been observed in cycling normal lymphocytes but was deficient in AS and PWS cells. We therefore investigated whether homologous pairing of 15q11-13 alleles occurs in normal cerebral cortical neurons and in vitro differentiated SH-SY5Y cells by fluorescence in situ hybridization (FISH). SH-SY5Y cells showed a significant increase in the percentage of 15 (CEP 15) and 15q11-13 (*GABRB3*) paired alleles following differentiation, coinciding with an increase in MeCP2 expression. In contrast, no significant increase with differentiation was observed in the percentage of chromosome 11 (CEP 11) or 22 (LSI 22) pairing, demonstrating that the pairing was not observed for another imprinted or acrocentric chromosome, respectively. A similar FISH analysis performed on normal human cerebral samples on a tissue microarray demonstrated a significant increase in homologous pairing in samples from individuals older than 18 months compared to younger infants that was also specific to chromosome 15 or but not 11. Significant deficiencies in the percentage of paired chromosome 15 alleles were observed in RTT, AS/PWS, and autism brain samples compared to normal controls. In contrast, no significant differences were observed between these populations using a chromosome 11 probe. As the RTT and autism samples also showed deficiencies in MeCP2 expression, these combined results suggest that these neurodevelopmental disorders may share an overlapping epigenetic pathway involving chromosome 15q11-13 and further suggest a potential role for MeCP2 in the nuclear organization of chromosomes in the developing human brain.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 280 from 2003 ASHG Annual Meeting

Functional and evolutionary annotation of the chromosome 17 centromere. *K. Rudd[1,2], J. Dunn[1], H.F. Willard[1,2].* 1) Dept Genetics, Case Western Reserve Univ, Cleveland, OH; 2) Institute for Genome Sciences and Policy, Duke Univ, Durham, NC.

The human centromere is a complex locus made up of megabases of tandemly repeated satellite DNA. As the site of kinetochore, the centromere is responsible for chromosome segregation. Despite its functional significance, assembly of satellite sequence in both the public and private efforts has been largely unsuccessful due to its highly repetitive nature. We have taken a genomic approach to identify and better understand the evolutionary and functional significance of the satellite present on chromosome 17. Higher-order satellite on chromosome 17 (D17Z1) is coincident with kinetochore proteins and is competent for centromere function based on its ability to form de novo centromeres in an artificial chromosome assay. However there are other types of satellite present on this chromosome that may or may not play a role in centromere activity. Unlike higher-order satellite that is highly homogeneous (98-99% identity among higher-order repeats), monomeric satellite lacks a higher-order periodicity, and its monomers are only 65-85% identical. On the q side of the centromere, we have constructed a ~500 kb contig containing monomeric satellite interspersed with various retrotransposon elements. On the p side, we have assembled a ~750 kb contig containing three regions of monomeric satellite that links D17Z1 to the euchromatin of 17p11.2. This contig includes a ~250 kb region of novel higher-order satellite that is two monomers shorter than and 92% identical to D17Z1. Phylogenetic analysis of the higher-order satellite and the four regions of monomeric satellite demonstrate that higher-order satellite is evolutionarily distinct from monomeric satellite, likely reflecting the recent emergence of higher-order organization in the primate lineage. Our analysis of the chromosome 17 centromere models satellite evolution as well as function. Ongoing experiments examine the multiple types of satellite present on chromosome 17 using artificial chromosome assays to further elucidate the characteristics of satellite required for centromere function.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

The breakpoint region of the most common isochromosome, i(17q), in human neoplasia is characterized by a complex genomic architecture with large palindromic low-copy repeats. *A. Barbouti[1], P. Stankiewicz[2], B. Birren[3], C. Nusbaum[3], C. Cuomo[3], M. Höglund[1], B. Johansson[1], A. Hagemeijer[4], S.-S. Park[2,5], F. Mitelman[1], J.R. Lupski[2,6,7], T. Fioretos[1].* 1) Department of Clinical Genetic, Lund University Hospital, Lund, Sweden; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Whitehead Institute for Biomedical Research/MIT Center for Genome Research, Cambridge, MA; 4) Department of Human Genetics, University of Leuven, Leuven, Belgium; 5) Department of Clinical Pathology, Seoul National University Hospital, Seoul, South Korea; 6) Department of Pediatrics, Baylor College of Medicine; 7) Texas Childrens Hospital, Houston, TX.

Although a great deal of information has accumulated regarding the mechanisms underlying constitutional DNA rearrangements associated with inherited disorders, virtually nothing is known about the molecular processes involved in acquired neoplasia-associated chromosomal rearrangements. Isochromosome 17q i(17q) is one of the most common structural abnormalities in human neoplasms and has been described both as a primary and a secondary chromosomal abnormality, indicating that it plays an important pathogenetic role in tumorigenesis as well as in tumor progression. We previously identified a breakpoint cluster region for i(17q)-formation in 17p11.2 and hypothesized that genomic architectural features could be responsible for this clustering. To address this hypothesis, we precisely mapped the i(17q) breakpoints in 11 hematologic malignancies and determined the genomic structure of the involved region. Our results reveal a complex genomic architecture in the i(17q) breakpoint cluster region characterized by large (~ 38-49 kb), palindromic, low-copy repeats (LCRs), strongly suggesting that somatic rearrangements are not random events but rather reflect susceptibilities due to the genomic structure.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Spectral Imaging for Phenotype Analysis of Cancer Cells. *H. Weier[1], L. Chu[1], Y. Ito[1], J. Hsu[1], J. Fung Weier[1,2], Y. Garini[3,4], N. Katzir[4], S. Hayward[5], P. Yaswen[1]*. 1) Life Sci Div, Lawrence Berkeley Lab, Berkeley, CA; 2) University of California, San Francisco, CA; 3) Dept. Imaging Science & Technology, Delft University of Technology; 4) Applied Spectral Imaging, Migdal HaEmek, Israel; 5) Dept. Urological Surgery, Vanderbilt University Medical Center, Nashville, TN.

Analyzing RNA transcript levels with cDNA micro-arrays provides a wealth of information. However, many solid tumors show heterogeneous expression of (onco)genes. The complex nature of tumor samples requires single cell-specific analysis techniques to test predictions from micro-array experiments. We developed a cRNA/cDNA fluorescence in situ hybridization (FISH) technique to measure cell-by-cell levels of multiple tumor markers with high spatial resolution. Our approach based on Spectral Imaging (SIm) and digital image analysis offers more resolution than filter-based microscopy and is based on simultaneously exciting fluorochromes in different wavelength intervals. Fixed wavelength bandpass filters in the microscopes emission path are replaced with an interferometer. A camera attached to the interferometer exit records series of resulting interferograms, while the optical path difference is changed stepwise. Spectral Images, i.e., stacks of images representing the image intensities as a function of the wavelength can then be calculated for all points in the image by subjecting interferograms to a Fourier transformation. Our set-up allows simultaneous excitation of six different dyes, and additional single wavelength filter combinations are used to record images from Spectrum Aqua- or Pacific Blue-labeled DNA probes or DAPI. We applied our novel FISH technology to analyze cell-by-cell expression of tyrosine kinase genes and genes involved in the stepwise immortalization of breast epithelium in finite life span, immortalized and transformed breast cell lines as well thyroid and prostate tumor tissues. We also profiled the expression of genes after grafting tumor cells in the sub-renal capsule of mice. Although costly, this break-through technology is easy to set up, and provides cell-by-cell expression pattern with sub-micron resolution.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 283 from 2003 ASHG Annual Meeting

Prognostic markers in CLL by array-based comparative genomic hybridization. *C. Lee*[1,2], *D. Drandi*[2,3], *P. Dal Cin*[1,2], *J. Gribben*[2,3]. 1) Pathology, Brigham & Woman's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Dana Farber Cancer Institute, Boston, MA.

In Chronic Lymphocytic leukemia (CLL), genomic aberrations are important independent predictors of disease progression and survival. The most common aberrations in CLL included gains of chromosomal material in chromosome 12 as well as losses in 13q, 11q, and 17p. Fluorescence in situ hybridization (FISH) has greatly improved the ability to detect known chromosomal aberrations. Cytogenetic and molecular findings provide important diagnostic, clinical, and prognostic information, which are increasingly being used to contribute to treatment decisions and follow-up of CLL patients.

Array-based comparative genomic hybridization (array-CGH) is a molecular cytogenetic method that provides a comprehensive and genome-wide view of chromosomal imbalances. Through this technology it is now possible to identify and measure DNA sequence copy number gains and losses to identify new genetic signatures that may predict survival and to improve our knowledge of the molecular pathogenesis of this disease.

We performed array-CGH analysis on 20 patients with CLL, all of whom had their disease previously characterized by FISH in the Clinical Cytogenetics Laboratory at Brigham and Women's Hospital. Array-CGH detected the cytogenetic abnormalities previously characterized by FISH, and detected a number of cryptic abnormalities, some of which were shared among patients. Correlating the array CGH findings with patient outcome data, we have identified at least one novel cryptic deletion which appears to have a high correlation with patient prognosis.

Copyright © 2003 The American Society of Human Genetics. All rights reserved.

Program Nr: 284 from 2003 ASHG Annual Meeting

Quantum dot probes for human metaphase chromosomes. *Y. Xiao, P. Barker.* DNA Technologies Group, Biotechnology Division, National Institute of Standards and Technology, 100 Bureau Drive, Mail Stop 831, Gaithersburg, MD 20899-8311.

Conventional organic fluorophores such as FITC and Texas Red have been used extensively as fluorescent labels for clinical cytogenetics FISH assays. During illumination, these compounds are subject to fading, and in multicolor applications, individual excitation and emission filters must be matched to each fluorophore. We have evaluated more quantitative labeling systems to improve sensitivity, photostability and quantitation of FISH data in connection with a new NIST Standard for HER2 testing. The electrical and optical advantages of a new class of inorganic fluorophores called semiconductor nanocrystals or quantum dots have held promise for biological labeling. These nanocrystallites contain hundreds to thousands of atoms, and their color properties depend primarily on crystal diameter. All colors can be excited by a single wavelength light, making them attractive for multiplexing applications. As a model system, we have compared detection of biotinylated DNA probes with streptavidin conjugates of fluorescein, Texas Red and Qdot605 in *in situ* hybridization (FISH) experiments with total human DNA and specific gene probes. We show that the quantum dot probes were (200-1100%) brighter than either organic fluorophores. In addition, in photostability/photobleaching experiments, organic fluorophore signal decreased to 10-27% of initial levels under continuous mercury lamp illumination over a 2 hr period. In similar conditions, the commercially available semiconductor nanocrystal Qdot605 probe decreased to 70% initial intensity. The signal flickering previously reported in unconjugated CdSe quantum dots was also observed here in nuclei hybridized with total biotinylated DNA. These results demonstrate that quantum dot conjugates improve probe photostability and brightness in FISH detection systems. (Supported in part by NIST HER2 SRM and the Office of Womens Health, NIH).

Copyright © 2003 The American Society of Human Genetics. All rights reserved.