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Identification of candidate genes involved in genitourinary malformations. J. Overhauser¹, E.R. Frizell¹, R. Dolsky¹, K. Rojas¹, R. Sutphen², ¹Thomas Jefferson University, Philadelphia, PA and ²University of South Florida, FL.

Cryptorchidism and hypospadias are the two most common abnormalities of the external genitalia in the male. Recent studies reveal a doubling of the frequency of these abnormalities in the last 20 years. In order to understand environmental influences that may be increasing the incidences, it is helpful to understand the genetic pathways that may be perturbed. Several chromosomal syndromes have genitourinary malformations within their clinical phenotype including the 18q-syndrome. Therefore, it is likely that a gene involved in genitourinary development maps to 18q. Recently, we have described a patient with an apparently balanced t(1:18)(q32;q22) karyotype with genitourinary malformations that are also observed in the 18q- syndrome. Clinical features in this patient include hypospadias, micropenis, and a labialized scrotum. We are testing the hypothesis that expression of a gene at or near the translocated breakpoint on chromosome 18 is disrupted by the translocation. A detailed physical map composed of BAC clones around the translocation breakpoint has been generated. Numerous ESTs that have been mapped to 18q21.3-q22.3 by the International Radiation Hybrid Consortium have been mapped relative to the translocation breakpoint and the BAC clones. Several candidate genes have been identified by this EST mapping. One gene produces two transcripts of 4.4kb and 7kb in size that differ by the length of their 3' untranslated region. This gene is expressed in testes as well as in other tissues and has previously been described as the human equivalent of the chicken cadherin 7 gene. Another cDNA that shows homology to OB-cadherin maps in the same vicinity as the cadherin 7 gene. Therefore, it appears that a cluster of cadherin genes maps near the site of the translocation breakpoint. Experiments are currently in progress to characterize this gene cluster and to investigate the role of these genes in genitourinary development.

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Recent progress in identification of the X-linked Spinal Muscular Atrophy (XL-SMA) gene confirms a major disease locus between Xp11.3-Xp11.2.
L.L. Baumbach¹, D. Dressman², A. Meindl¹ and E. Hoffman¹. ¹Univ. of Miami Sch of Med., Miami, FL., ²Univ. Of Pittsburgh Sch of Med, Pittsburgh, PA and ³Ludwig Maximilians University, Munchen, Germany.

We report our recent efforts toward the identification of the X-linked lethal infantile spinal muscular atrophy (XL-SMA) locus. Our collaborative group has previously described several families which display an X-linked recessive form of human motor neuron disease (*AJHG*, 61S, #1554, 1997). Defining features include multiple generations of males with congenital onset of severe hypotonia, contractures and fractures; muscle biopsy indicative of neurogenic atrophy, and autopsy indicating loss of anterior horn cells. The first family analyzed for linkage mapped to Xp11.3-q11.2, and multipoint analysis refined this interval to a 11 cM region defined by DXS993-DXS991, with a maximum LOD score of 2.63 (*Hum Molec Genet.*, 4(7):1213-16,1995). Subsequently, seven unrelated families (six N. American, one European) have been identified and ascertained. These families were selected based on the above described inclusion criteria. Genomic DNA was prepared from blood and/or tissue specimens from affected and unaffected males, and obligate carrier females, and analyzed using X-chromosome highly polymorphic DNA repeats. Concordance analysis was used to define maternal meiotic recombination breakpoints surrounding a disease gene region, followed by multipoint linkage analysis using additional DNA markers. Results of these studies were interpreted using the *LINKAGE* and *LITESSE* programs, using the assumptions of a completely penetrant disease, with a gene frequency of 1×10^{-6} . These combined results indicated that in addition to the first family reported above, four additional families have been linked to the same candidate disease gene interval, Xp11.3-q11.2. Multipoint linkage analysis has been completed for three of these four families and has refined this interval to a region defined by DXS991-DXS1003, with a maximum LOD score of 2.73 for these three families. The total additive LOD score for these three families, and the family previously published, is 5.36 at a *Theta* of 0.00. **These results strongly support the existence of a major disease locus for XL-SMA between DXS 1003-DXS 991 (Xp11.3-Xp11.2).** We are attempting to narrow the disease gene region through a search for recombinant individuals in each of the families using additional polymorphic markers. We are also focusing our efforts on mutation screening of candidate disease genes within this region, particularly new candidates identified from a recently completed 1.1 mB transcript map of Xp11.3. Latest findings will be presented.

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Exclusion of linkage to chromosome 3q in some familial cases of the Cornelia deLange Syndrome. I.D. Krantz¹, B.P. Conti¹, M. Hofreiter¹ and L. Jackson². ¹Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia and The University of Pennsylvania School of Medicine, and ²The Division of Medical Genetics, Jefferson Medical College, Philadelphia, PA.

The Cornelia deLange Syndrome (CDLS) (OMIM #122470) is a complex genetic developmental disorder consisting of characteristic facial features, hirsutism, various ophthalmologic abnormalities, abnormalities of the upper extremities, gastroesophageal dysfunction, growth and neurodevelopmental retardation. Most cases of CDLS appear to be sporadic. Familial cases are rare and demonstrate autosomal dominant inheritance.

Several patients with CdLS have had chromosomal abnormalities, suggesting potential genomic regions within which the disease gene(s) may lie. Partial phenotypic overlap between CdLS patients and patients with duplication of chromosome 3q26-27 has been noted. A patient described by Ireland et al (*J Med Genet* 28:639-640, 1991) with an apparently balanced translocation with a breakpoint within the dup3q critical region and classic CdLS phenotype added further support to the hypothesis that a CdLS gene lay within this chromosomal region. It has been postulated that a gene within the duplicated region on chromosome 3q is deleted or mutated in patients with CdLS and results in a different but mildly overlapping phenotype.

We have performed linkage analysis in 9 familial cases of CdLS to the minimal critical region for the dup3q syndrome that encompasses the translocation breakpoint on chromosome 3q. 12 markers spanning approximately 40 Mb (37 cM) were used to haplotype the nine families and linkage analysis performed. In 4/9 families (44%) the affected sib pairs did not share haplotypes to this region from either parent while in the remaining 5 families (56%) at least one parental allele was shared. These studies indicate that chromosome 3q26-27 does not segregate with the CdLS phenotype in all familial cases studied. This would imply that this region on chromosome 3 may not be associated with CdLS or may be associated with a subset of CdLS cases. Other candidate loci are being examined for linkage to CdLS which may prove to be a genetically heterogeneous disorder.

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Characterization of a novel candidate gene in Xp22.3 with homology to *Drosophila msl3*. I.B. Van den Veyver¹, S. Prakash¹, B. Franco³ and H.Y. Zoghbi^{1,2}. ¹Baylor College of Medicine and ²Howard Hughes Medical Institute, Houston, TX, and ³T.I.G.E.M., Milan, Italy.

MLS syndrome and orofaciocigital syndrome type I (OFD1), two X-linked dominant, male-lethal disorders featuring brain, eye, kidney and limb malformations, are caused by deletions of Xp22.3 (MLS) or mutations in a closely linked gene (OFD1). Three loci for non-syndromic mental retardation (MRX) were also mapped to the same region. These observations suggest that Xp22.3 may contain gene(s) which are essential for brain, eye, kidney and limb development. In an effort to identify candidate genes in Xp22.3, we have analyzed more than 1 Mb of genomic sequence. Here we report the characterization of a novel gene, named *homolog of msl3* (*HMSL3*), which was identified by sequence analysis of BAC GS-590J6 (GenBank AC004554). BLAST homology searches with this sequence led to the identification of 25 overlapping human EST clones and 2 mouse EST clones. We derived a consensus cDNA sequence containing a 1257 bp ORF, which encodes a 462 amino acid protein. Two distinct regions share 40% amino acid identity with the *Drosophila male-specific lethal 3* (*Msl3p*) protein: a chromo domain and a putative leucine zipper motif at the C terminus. *HMSL3* spans 20 Kb of genomic DNA and contains 12 exons transcribed from telomere to centromere. It is expressed as a ubiquitous 2.5 Kb transcript with a 2.8 Kb isoform in skeletal muscle. *Drosophila msl3* participates in a sex-specific dosage compensation pathway as part of a multiprotein complex, which binds to and activates transcription from the single X chromosome in males. As a putative chromo domain transcription factor, *HMSL3* is an excellent candidate to cause the developmental defects in *OFD1* patients, and in patients with MLS-like features who do not have large deletions, as in Aicardi syndrome and Goltz syndrome. Primers were designed to amplify each exon from genomic DNA. 24 Aicardi, 3 Goltz and 2 *OFD1* patients were screened for mutations in *HMSL3* using heteroduplex analysis and sequencing. To date no mutations have been found. In an effort to obtain full-length cDNAs for *HMSL3*, 57 positive clones were identified from a human fetal kidney library. Several cDNAs include a novel alternatively spliced exon (1a) with additional homology to *Drosophila msl3*. Further characterization of the isoforms and additional mutation analysis are in progress.