Sixth Structural Birth Defects Meeting
The Conference Center at the Maritime Institute
Linthicum Heights, MD

May 13-14, 2008

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NICHD SIXTH STRUCTURAL BIRTH DEFECTS MEETING
AGENDA

Monday, May 12, 2008

Arrival  Check in (after 3 pm) to the Conference Center at the Maritime Institute
5:00-7:30 pm  Dinner on your own – Main Dining Room

Tuesday, May 13, 2008

6:30 am  Breakfast – Main Dining Room opens
9:00  Welcoming remarks (Bridgeroom)
9:15  Keynote Address – Jonathan Gitlin, M.D., Washington University School of Medicine, St. Louis
Mechanisms of gene-nutrient interaction essential for early human development
10:15  Break – Beverages and continental breakfast in break station area
10:30  Ian Krantz, M.D.
Cornelia de Lange Syndrome and the cohesinopathies: Developmental repercussions of cohesin dysfunction
10:50  Arthur Lander, M.D., Ph.D.
Vertebrate animal models of Cornelia de Lange Syndrome
11:10  Dale Dorsett, Ph.D.
A Drosophila model for Cornelia de Lange Syndrome
11:30  General questions and discussion
12:00-1:00 Lunch – Main Dining Room
1:00  Robert Greene, Ph.D.
Transcriptional coactivators and pregnancy outcomes
1:20  Mike Bamshad, M.D.
Congenital contractures
1:40  Jacqueline Hecht, Ph.D.
Genetic study of HOXA, HOXD D and IGFBP3 genes in isolated clubfoot
2:00  Carol Wise, Ph.D.
Genetics of idiopathic scoliosis: Insights into a common and complex disease of childhood

2:20  General questions and discussion

2:40  Break – coffee and snacks in the break station area

3:00  Dave Rimoin, M.D., Ph.D.
The International Skeletal Dysplasia Registry

3:20  Bill Wilcox, M.D., Ph.D.
Improving the management of achondroplasia and developing novel therapies

3:40  Dan Cohn, Ph.D.
Cartilage gene expression and the skeletal dysplasias

4:00  Deborah Krakow, M.D.
The bent bone dysplasias

4:20  Brendan Lee, M.D., Ph.D./Presented by Dave Rimoin
Skeletal dysplasias

4:40  Simon Rhodes, Ph.D.
The LHX3 transcription factor gene in pituitary development and pediatric hormone deficiency diseases

5:00  Becky Burdine, Ph.D.
Left-right patterning and cardiac morphogenesis in zebrafish

5:20  General questions and discussion

6:00-7:30  Dinner – Main Dining Room

Wednesday, May 14, 2008

6:30 am  Breakfast – Main Dining Room opens

9:00  Peggy Honein, Ph.D., M.P.H.
An update on the National Birth Defects Prevention Study

9:20  Tony Scarpa, M.D., Ph.D., Director, Center for Scientific Review, NIH
CSR review of structural birth defects research applications

10:00  Town Hall Meeting

Coffee and snacks available in the break station area throughout morning
10:30  Pat Donahoe, M.D./Presented by Barbara Pober, M.D. and Kasper Lage, Ph.D.
Update on “Gene mutation and rescue in human diaphragmatic hernia”

10:50  Daryl Scott, M.D.
Identifying genes for congenital diaphragmatic hernia

11:10  Anne Slavotinek, M.D., Ph.D.
Microarray studies in a male with congenital diaphragmatic hernia and anophthalmia reveal a novel 18q22.1 deletion

11:30  General questions and discussion

12:00  Lunch and Departure
TUESDAY ABSTRACTS
Mechanisms of gene-nutrient interaction essential for early human development

The relevance of nutrition during development is revealed by recent data highlighting the importance of folate intake before and during pregnancy for preventing neural tube defects. Despite these observations, the metabolic factors that place specific pregnancies at higher risk remain unknown. Copper is an essential nutrient that plays a critical role in the biochemistry of normal development. In Menkes disease, an inability to acquire copper \textit{in utero} due to inherited loss-of-function mutations in the gene encoding a copper-transport ATPase (\textit{Atp7a}), results in skeletal defects, cerebellar degeneration, and severe failure to thrive. Utilizing a chemical genetic screen, we identified small molecules that perturb copper homeostasis in zebrafish. Our findings reveal a role for copper in hematopoiesis, brain development and notochord formation and demonstrate a hierarchy of copper metabolism within the embryo. A genetic screen for embryos phenocopied by copper deficiency, identified \textit{calamity}, a mutant defective in the zebrafish ortholog of \textit{Atp7a}. The gene dosage of \textit{Atp7a} determines the sensitivity to copper deprivation, revealing that the observed developmental hierarchy of copper metabolism is informed by specific genetic factors and suggesting that suboptimal copper metabolism may contribute to birth defects. Consistent with this concept, beta-aminopropionitrile, a known inhibitor of the copper-dependent lysyl oxidases, causes notochord distortion in the zebrafish embryo identical to that seen in copper deficiency and partial knockdown of specific lysyl oxidases markedly sensitizes developing embryos to notochord distortion if copper availability is diminished. A forward genetic screen for zebrafish mutants that exhibit notochord sensitivity to lysyl oxidase inhibition identified \textit{puff daddy}^{gw1} (\textit{pfd}^{gw1}), resulting from disruption of the gene encoding the extracellular matrix protein fibrillin-2. Identification of a genetic interaction between fibrillin-2 and the lysyl oxidases in late notochord formation reveals a complex interplay of gene expression and nutrient availability critical to notochord development and provides insight into specific genetic and nutritional factors that may play a role in the pathogenesis of structural birth defects of the axial skeleton. Having described the gene-nutrient interactions of copper metabolism and \textit{atp7a} during development, our findings demonstrate the potential of chemical and genetic screens to elucidate the metabolic and genetic interplay of other nutrients that may provide insight into the roles of suboptimal nutrition and genetic variation in human birth defects.
Ian D. Krantz, M.D.
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Cornelia de Lange Syndrome and the cohesinopathies: Developmental repercussions of cohesin dysfunction

The cohesin proteins compose an evolutionarily conserved complex whose fundamental role in chromosomal cohesion and coordinated segregation of sister chromatids has been well characterized across species. Recently regulators and structural components of cohesin have been found to surprisingly cause specific human developmental disorders (collectively termed “cohesinopathies”) when mutated. Mutations in NIPBL, the vertebrate homolog of the yeast sister chromatid cohesion 2 (Scc2) protein, a regulator of cohesin loading and unloading, are responsible for approximately 50% of cases of Cornelia de Lange syndrome (CdLS). Mutations in the cohesin structural components SMC1A and SMC3 were also found to result in CdLS. CdLS is a multisystem developmental disorder classically characterized by facial dysmorphia, upper extremity malformations, hirsutism, cardiac defects, growth and cognitive retardation, and gastrointestinal abnormalities. A mild form of CdLS has been consistently reported, however, it had not been clear if this is a distinct etiologic entity from classic CdLS or truly a mild manifestation, however molecular testing of cohesin genes has identified mutations in individuals with very subtle features of CdLS bordering on apparent isolated mental retardation. Mutations in another cohesin regulator, ESCO2, result in Roberts syndrome (RBS) and SC phocomelia. Roberts syndrome is a recessively inherited multisystem disorder with craniofacial, limb, cardiac, other systemic abnormalities and neurocognitive dysfunction. While there is some overlap between Roberts syndrome and CdLS they are clinically readily differentiated. Other developmental disorders have also recently been found to be associated with cohesin dysfunction. The recent implication of the cohesin complex and its regulators in transcriptional control has shed light on the mechanism by which alterations in this complex leads to the specific phenotypes seen in these disorders. A review of cohesin function, the disorders associated with disruption of this pathway and future clinical and bench-top research directions will be discussed.
Vertebrate animal models of Cornelia de Lange Syndrome*

Cornelia de Lange Syndrome (CdLS) is characterized by structural and functional abnormalities in cardiopulmonary, gastrointestinal, skeletal, nervous and other systems. Genetic alterations that cause CdLS have so far been discovered in three genes that encode structural or regulatory components of cohesin, a protein complex initially characterized for its role in mediating sister chromatid cohesion. Haploinsufficiency for one of these genes, NIPBL, accounts for the vast majority of CdLS of known genetic origin, and at least 50% of all CdLS. To gain insight into how NIPBL deficiency gives rise to CdLS, and how diagnosis and treatment of this syndrome might be improved, we developed two animal models of Nipbl deficiency: Mice heterozygous for a loss of function mutation in Nipbl were created using a gene trap embryonic stem cell line. Such mice exhibit many characteristic of CdLS, including growth retardation, microbrachycephaly, craniofacial abnormalities, abnormal hearing, heart defects, and seizures. Three quarters of these animals die within the first three weeks of life. Zebrafish were made deficient in expression of their two Nipbl paralogues (zNipbl1 and zNipbl2) through the injection of antisense morpholino oligonucleotides (MO) into fertilized eggs. Such fish exhibit a variety of developmental abnormalities depending on the degree of reduction of Nipbl expression. In both animal models, mRNA expression profiling has provided insights into pathogenesis. In mouse, expression data from embryonic brain suggest that differentiation of GABA-ergic neurons is diminished in Nipbl mutants, and immunohistochemical and in situ hybridization analysis of adult brains revealed a marked reduction in the numbers of such neurons in the cerebral cortex. This abnormality may explain why these mice develop seizures. In fish, expression data from gastrulation-stage embryos suggest that Nipbl-deficiency impairs endoderm differentiation. Subsequent analysis revealed specific early abnormalities in gut and heart development that are consistent with an endodermal defect. These observations immediately suggest hypotheses for the origins of heart and gut defects in CdLS, and we hope to test these hypotheses shortly in the mouse. Expression array data also indicate that the chromosomai environments of many of the genes that are misregulated in response to Nipbl share intriguing structural features, including spatial clustering, proximity to odorant and taste receptors, and regulation by known locus control regions. The significance of these features will be discussed in light of recently published studies demonstrating a role for cohesin in the control of transcriptional insulation. Supported by grants from the NIH (P01 HD052860 to ADL, ALC, and TFS); the CdLS Foundation USA; and the Center for Hearing Research at the University of California, Irvine.

* with Shimako Kawauchi1,3,4, Rosaysela Santos1,3, Martha E. Lopez-Burks2,3, Trevor Hoffman2,3, Akihiko Muto2,3, Clint M. Young1,3, Michelle P. Hoang2,3, Abigail Chua2,3, Leonard M. Kitzes1,4, Taotao Lao5, Mark S. Lechner5, Benedikt Hallgrimsson6, Thomas F. Schilling2,3, and Anne L. Calof1,3,4.

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Cornelia de Lange syndrome (CdLS) is characterized by slow growth, mental retardation, and structural birth defects in limbs and organs. CdLS is caused by mutations affecting proteins required for sister chromatid cohesion (Krantz et al. 2004; Tonkin et al. 2004; Musio et al. 2006; Deardorff et al. 2007). Current data, however, argue that the developmental deficits are largely caused by changes in gene expression, and not by defects in chromatid cohesion.

About half of CdLS patients have heterozygous loss-of-function mutations in Nipped-B-Like (NIPBL), and some 5% have missense or small in-frame deletion mutations affecting the Smc1 and Smc3 subunits of the cohesin complex. Cohesin is the glue that holds sister chromatids together, and NIPBL is required for cohesin to bind to chromosomes. The leading idea is that cohesin, which has a ring-like structure, encircles the sister chromatids to hold them together.

We discovered Nipped-B, the Drosophila homolog of NIPBL, in a genetic screen for factors that regulate the expression of the cut and Ultrabithorax (Ubx) homeobox genes (Rollins et al. 1999). Heterozygous loss-of-function Nipped-B mutations reduce cut and Ubx expression. The same mutations are lethal and cause severe sister chromatid cohesion defects when homozygous. Heterozygous mutants, however, do not cause cohesion defects.

Reducing the dosage of cohesin subunits has the opposite effect as Nipped-B mutations, and increases cut gene expression in the developing wing margin, leading us to hypothesize that cohesin inhibits cut gene expression, most likely by interfering with communication between the promoter and a distant wing margin enhancer (Rollins et al. 2005; Dorsett et al. 2005). We hypothesize that Nipped-B dynamically controls cohesin binding to regulate cut activation.

To gain further insights into how Nipped-B and cohesin regulate genes we used chromatin immunoprecipitation with tiled microarrays to map their binding genome-wide in cultured cells (Misulovin et al. 2008). Nipped-B and cohesin co-localize genome-wide, and they bind preferentially to active genes. There is also significant overlap with RNA polymerase II binding. We identified nearly 500 genes that bind cohesin, most of which are actively transcribed. About 100 of these genes bind cohesin in only one cell type, and there is a 20-fold higher probability that there is elongating RNA polymerase on the gene when cohesin binds.

One of the active genes that binds cohesin through its entire transcribed region encodes the steroid hormone receptor (EcR). Recently Schuldiner et al. (2008) discovered that EcR is underexpressed in postmitotic neurons that lack cohesin, causing an axon pruning defect. This suggests that cohesin directly facilitates EcR transcription, which is opposite to its effect on cut.

We currently theorize that transcription unravels chromatin to fit into the 35 nm diameter of the cohesin ring, and that cohesin then has multiple effects on transcription, including interfering with enhancer-promoter interactions and facilitating elongation by RNA polymerase. We also posit that Nipped-B dynamically regulates cohesin binding to mitigate the negative effects and facilitate the positive effects of cohesin on transcription.

A woman’s health plays a major role in the outcome of her pregnancy. To give birth to a healthy, thriving baby, the nutritional value of a woman’s diet is critical. Folate, a water-soluble B vitamin, is required for a pregnant woman’s increasing blood supply, the growth of fetal tissues, and normal development of the craniofacial complex. Maternal folate status is one condition that has a profound influence on development of the CNS, and the incidence and recurrence of neural tube defects (NTDs). Extant data argue for interactions between folate status and developmental pathways controlled by specific transcriptional regulators. The overall hypothesis our studies address is that normal CNS development in the mammalian embryo requires folate-mediated activation of a transcriptional complex, functionally dependent on proper expression and integration of specific transcriptional coactivators. Specifically, we propose that not only is proper expression of Folbp and transcriptional coactivators such as CBP, p300, Cited2, Cart1, and AP-2 requisite for CNS formation, but the integration of these molecules into a functional regulon is critical to normal CNS morphogenesis. Noteworthy is the fact that mutations in each of the transcriptional co-activator genes - CBP, p300, Cited2, and Cart1 result in NTDs in mice.

Preliminary data to date: 1) CBP, p300, Cited2, and Cart1 were expressed in the murine dorsal neural folds on gd 8.5 and subsequently exhibited temporal- and tissue-specific patterns of expression during NT development; 2) microarray analysis of RNA derived from gd 9.5 folbp1/- and wild type embryos revealed that alteration in folate nutritional status had a dramatic effect on the expression levels of numerous nuclear transcription factors; 3) when administered to pregnant folbp1+/- dams bred to folbp1+/- sires, 40 mg/kg folic acid rescued 100% of the nullizygous embryos from exencephaly, orofacial clefts and prenatal lethality; 4) In vitro siRNA inhibition of the synthesis of CBP and p300 revealed that both coactivators behave as tumor suppressors in cells derived from the 1st branchial arch; and 5) utilizing DNA obtained from 25 nonHispanic white infants with spina bifida and 25 nonHispanic white infants without any birth defects (controls), analysis of genetic variation within the Cited2 gene using a commercially available SNP array provided data on 134 SNPs associated with Cited2. While the limited number of study subjects to date does not confirm associations, the numerous SNPs identified offers compelling possible associations with risk for spina bifida. Moreover, one of the SNPs (rs 1131431) was previously shown to be associated with elevated risk for spina bifida.

Lastly, diminished folate status is also associated with exposure to cigarette smoke. Given that the state of Kentucky has the highest prevalence of pregnant women who smoke, it may not be coincidental that the frequency of spina bifida in Kentucky greater than the national average. We have thus established an animal model of cigarette smoke-induced in utero growth retardation and abnormal development with which to investigate the potential impact of maternal and fetal folate status on cigarette smoke-induced abnormalities.

*With Gary Shaw, March of Dimes Birth Defects Foundation, Calif. Birth Defects Monitoring Program, Berkeley, CA and Rick Finnell, Inst. of Biosci. & Technol., Texas A&M University, Houston, TX
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Congenital contractures

The overall goal of this project is to characterize the genetic and molecular basis of congenital contractures. Worldwide more than 100,000 children are born each year with a congenital contracture such as clubfoot, and there is estimated to be more than 5,000,000 people living with congenital contractures. Despite exhaustive research, the etiology and pathogenesis of most congenital contractures remains unknown. Over the past decade, we have used a group of monogenic syndromes characterized by congenital contractures called the distal arthrogryposes (e.g., Freeman-Sheldon syndrome, Sheldon-Hall syndromes) as a model system to identify genes involved in the pathogenesis of contractures. To date, we have identified mutations in 7 genes that cause different distal arthrogryposis syndromes. All of these genes encode components of the troponin-tropomyosin complex of fast-twitch myofibers and are expressed primarily in the peripheral muscles of the limbs. These results suggest that congenital contractures represent a new class of muscle disease caused by perturbation of contractile proteins during limb development. Current studies are underway to document the muscle morphology and myofiber contractility of affected individuals with the most common of these mutations. These studies will provide us with an opportunity to directly study the pathogenesis of congenital contractures and to consider novel therapeutic interventions. This project will contribute to our understanding of congenital contractures, the physiology of skeletal muscle, and human limb development.
Genetic study of HOXA, HOXD D and IGFBP3 genes in isolated clubfoot*

Clubfoot, also known as, idiopathic talipes equinovarus (ITEV) is a common birth defect occurring in 1/1000 livebirths. The etiology of clubfoot is complex with both genes and environmental factors causally implicated. We have used a candidate gene approach to identify the genes contributing to the development of clubfoot. Six chromosomal deletion regions are associated with syndromic clubfoot, including 2q31-33. This region contains the HoxD gene cluster, which is involved in axial and limb patterning. The HoxA gene cluster is also involved in limb formation, and mutations in both HoxA and HoxD are known to cause limb defects and syndromes with limb malformations. For example, the HoxD10 M319K mutation was found in a patient with a syndromic clubfoot. Interestingly, HoxA13 null mice have increased expression of IGFBP3, which is involved in apoptosis, a process that we have shown to be associated with clubfoot. This study was undertaken to determine whether variation in HoxA, HoxD and IGFBP3 genes are associated with ITEV. The study population consisted of nonHispanic white and Hispanic multiplex and simplex families. Twenty-one SNPs spanning the HoxA and HoxD clusters, including the M319K mutation and 12 SNPs spanning the IGFBP3 gene were genotyped. The M319K mutation was not present in any cases or 144 controls. SNPs in HoxA were significantly overtransmitted particularly those upstream of HoxA9 and involved in limb development. This result was confirmed in a secondary sample set. Several pairwise haplotypes were also overtransmitted with most of the significant haplotypes involving SNPs in regions expressed in the limbs. Six SNPs in IGFBP3 and many pairwise haplotypes were also significantly overtransmitted. Previously, we showed that mitochondrial-mediated apoptotic genes were associated with clubfoot. Linear regression model was used to detect gene-gene interactions between variants in apoptotic, Hox and IGFBP3 genes. Many gene-gene interactions were detected between Hox and apoptotic genes, most notably between the Hox genes, IGFBP3, Bid, and Casp3 in the nonHispanic white population and Hox, IGFBP3 and Apaf1 in the Hispanic population. These results suggest a biologic model for ITEV that involves the interaction of genetic variants in Hox and apoptotic genes, which individually may not be sufficient to cause clubfoot.

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Genetics of idiopathic scoliosis: Insights into a common and complex disease of childhood*

Idiopathic scoliosis (IS) is the most common pediatric spinal deformity, affecting ~3% of children worldwide. IS significantly impacts national health in the U.S., creating severe disfigurement and disability for over 10% of patients and costing billions of dollars annually for treatment. Clinical and population studies support strong genetic contributions to IS. We estimated sibling risk ratios for IS that are comparable to other well-described complex genetic diseases. Prior linkage studies of IS have suggested many chromosomal regions potentially underlying disease susceptibility. In a follow-up study of a linked 8q11q12 region we demonstrated association of IS with multiple loci in the CHD7 gene, the first candidate gene for IS susceptibility. Altogether these observations suggested that IS may be particularly amenable to haplotype-based, genome-wide methods of disease gene identification. Toward this end we have systematically ascertained IS patients treated at major U.S. pediatric orthopaedic centers. DNA samples from these patients will be used in a three-stage genome-wide association study (GWAS). Stage 1 will utilize 700 IS family trios, with follow-up (Stage 2) for most significant loci in a second cohort of 1,400 trios. Most promising ~2% of replicated loci will be assessed in a third, independent cohort of 1,000 trios ascertained throughout the U.S. De novo mutations and common copy number variation also will be assessed. We expect that these studies will reveal IS susceptibility loci in genes with strong effects across patient populations, and may provide supporting evidence for previously observed linkage peaks. Results should provide new biologic insights into IS susceptibility that will aid better clinical management and the development of alternative treatments.

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The International Skeletal Dysplasia Registry

The International Skeletal Dysplasia Registry was established over 40 years ago and currently has over 15,000 cases in a secure, web-accessible, computerized database with an anonymous code for each individual. The Registry is the largest resource in the world for research on the skeletal dysplasias and is the backbone of the research program. The materials accumulated include clinical information, radiographs, fixed and frozen chondro-osseous tissue samples, histology slides, electron micrographs, cultured fibroblasts and chondrocytes, lymphoblastoid cells lines, and DNA. Radiographic and chondro-osseous morphologic findings are coded in the database in a searchable format for rapid identification of cases with similar findings. Cases entered into the Registry include patients seen in our Genetics clinics as well as cases submitted from around the world. The materials obtained by the Registry are extensively used by all the Projects as well as by other collaborators.

The International Skeletal Dysplasia Registry, which serves as a core facility for this program project, has continued to grow as a source of clinical, radiographic, morphologic, biochemical, and molecular materials for our research in these disorders and for collaboration with other workers. The Registry has continued to expand and serve the other projects of this grant, as well as numerous other collaborators. From 2/1/07 to 1/31/08, we collected radiographs on 654 cases, histology specimens on 127, electron microscopy specimens on 70, frozen specimens on 76, LBL lines on 106, cultured fibroblasts on 64, cultured chondrocytes on 65, and DNA on 191 individuals. The total number of cases in the Registry is now over 15,000. We have provided families and materials to numerous investigators for collaborative studies. Although most patients first come to the Registry’s attention for help in diagnosis and management, patients are offered the ability to participate in the Registry’s research program and then asked to provide informed consent if they so desire. Radiographic images continue to be scanned and entered into the database. Images can be searched for by diagnosis, image type, view, procedure, and patient age at the time the image was obtained.

The International Skeletal Dysplasia Registry is the backbone of this program project and provides all human clinical and biological materials to each of the component research projects and other collaborators. It also provides the computerized web-based database used by all projects for retrieval of cases, clinical information, the ability to search for cases that might share a common molecular or pathogenetic basis, and to record research findings.
Improving the management of achondroplasia and developing novel therapies

Achondroplasia (ACH) is the most common non-lethal human dwarfing condition, affecting approximately 1 in 10,000 individuals. ACH is an autosomal dominant condition, but most cases are due to de novo activating mutations in the fibroblast growth factor receptor 3 gene. In addition to short stature, ACH has a narrow foramen magnum that can cause spinal cord compression and spinal canal stenosis frequently causing symptomatic claudication in adults.

Criteria for surgical decompression of the foramen magnum vary significantly between centers. We have recently identified several children with symptomatic spinal cord compression in the flexed position that is not present in the neutral position. Surgical decompression relieved the symptoms. We now advocate obtaining MRI in flexion and extension along with CSF flow studies for symptomatic patients.

The etiology of the spinal stenosis and small cranial base is, in part, due to poor endochondral bone growth. Studies from transgenic mice suggest that there is premature fusion of the synchondroses. Radiographic and histologic examination of synchondroses from cases of thanatophoric dysplasia, a more severe relative of ACH due to more potent activating mutations in FGFR3, also demonstrates premature fusion. If premature fusion occurs in ACH, it could limit the efficacy of growth promoting therapies under development.

Currently, the only effective treatment for increasing height in ACH is surgical limb-lengthening. Developing new therapies for ACH requires a thorough understanding of FGFR3 signaling and how it interacts with other signaling pathways to decrease growth. Through the work in our lab and others, simple models of the past have evolved into a much more complex system, with many important components remaining poorly understood. However, much of the impairment of growth in ACH is due to prolonged and excessive activation of ERK MAPK. This, in turn, results in slowed chondrocyte proliferation, altered cellular morphogenesis and differentiation, decreased extracellular matrix synthesis, and increased matrix degradation.

Many of the effects of ERK MAPK can be countered by the actions of C-natriuretic peptide (CNP) signaling. We are collaborating with a biotechnology company to develop a CNP analogue with a longer half-life that could be used to treat ACH. In addition, using an in vitro assay that we developed, we are testing small molecule libraries for their ability to counter the effects of FGFR3 activation. Since FGFR3 is one of the major negative regulators of endochondral bone growth, pharmacologic treatments effective for ACH may also promote growth in other dwarfing conditions. Agents countering FGFR3 actions could also be useful in cancers where FGFR3 acts as an oncogene.
Cartilage gene expression and the skeletal dysplasias*

The chondrodysplasias comprise a subset of 187 skeletal dysplasias that primarily affect cartilage. Among these disorders, 61 disease genes have been identified in 99 of the conditions. Thus for about 47% of the recognized chondrodysplasias, disease genes and mechanisms have yet to be identified. Of the 99 chondrodysplasias in which the disease gene is known, 34 of them result from a mutation in a gene with a cartilage-selective expression pattern. Using human fetal cartilage gene expression profiles and quantifying the cartilage selectivity of all genes expressed in the tissue, the genes in linked intervals for two chondrodysplasias were ranked by cartilage-selectivity to prioritize positional candidates and identify the disease genes. In an autosomal dominant form of brachyolmia, linkage studies localized the disease gene within an 11 Mb interval on chromosome 12. TRPV4, which encodes a calcium-permeable cation channel, was the most highly cartilage-selective gene in the interval and point mutations were identified in two families. Patch clamp studies demonstrated that the mutations activate the channel, suggesting that increased intracellular calcium dysregulates chondrocyte function to produce the phenotype. In an ancestrally consanguineous family with a new recessive spondyloepimetaepiphyseal dysplasia phenotype, homozygosity mapping localized the disease gene to a 17 Mb interval on chromosome 15. There were two cartilage-selective genes in the interval and homozygosity for a structural mutation predicted to alter the C-type lectin domain of aggrecan was found in the family. This region of the molecule mediates interactions between aggrecan and other extracellular matrix molecules, suggesting that the extreme short stature that characterizes the disorder results from disruption of these interactions. Using cartilage gene expression in large genetic intervals defined by linkage is an efficient way to identify chondrodysplasia disease genes, and the approach should be useful for other disorders in which there is a tissue-selective phenotype.

*with Deborah Krakow, M.D.
Deborah Krakow, M.D.
Medical Genetics Institute, Cedars-Sinai Medical Center and Departments of Human Genetics and Pediatrics, David Geffen School of Medicine at UCLA

The Bent Bone Dysplasias*

Interest in long bone bowing or angulation/bending of the femur dates back to 1947 when Caffey first reported on patients with congenital bowing of the femur. Despite many case reports describing radiographic evidence of bent or angulated femurs, it was not until 1971 when Maroteaux described campomelic dysplasia that the first distinct disorder was delineated. The current Nosology of Constitutional Disorders of Bone (2006) recognizes three bent bone disorders, campomelic dysplasia, Cumming syndrome and kyphomelic dysplasia, and suggests that many additional skeletal disorders can present with bent bones in the newborn period. To characterize the large group of disorders associated with bent femurs, with a goal of determining the disorders in which this finding is consistent enough to use for diagnostic purposes, a database search of the International Skeletal Dysplasia Registry identified more than 800 cases in which congenital angulation or bending of the femurs was found. Review of the radiographic findings in these cases, showed that more than 40 disorders have either bent femurs alone or a group of bones that appear bent or bowed on radiographs. Among these disorders, 66% of the cases were from three diseases; campomelic dysplasia (24.4%), thanatophoric dysplasia (23.9%) and osteogenesis imperfecta (18.1%). In addition to these, other entities with angulated femurs as a distinctive finding included Stuve-Wiedemann syndrome, cartilage hair hypoplasia, the perinatal type of Caffey disease, Antley-Bixler syndrome and Cumming syndrome.

Novel disorders were also identified within the cohort of unclassified bent bone dysplasias. In one such disorder, prenatal ultrasound evaluation showed poor mineralization of the calvarium, flattened facies, micrognathia, and bilaterally angulated femurs. Postnatal evaluation showed dysmorphic findings including hypertelorism, micrognathia, posteriorly rotated ears, erupted teeth, and an extremely short neck. Radiographic findings included absent mineralization of the calvarium and facies, hypoplastic calvicles, scapulae and pubis, bilaterally angulated long bones, and a distinctive brachydactyly. Histologic analysis of the growth plate showed relatively normal reserve, proliferative and hypertrophic zones. Trabecular bone appeared normal, but the peristeme was markedly thickened, irregular and hypercellular. Review of the database revealed six other cases with the same constellation of findings. Among the cases, there was no parental consanguinity and there were no familial recurrences, consistent with new dominant mutations producing the disorder.

*with William W. Wilcox, Ralph S. Lachman and David L. Rimoin
Autosomal dominant osteogenesis imperfecta (OI) is caused by mutations in the genes (COL1A1 or COL1A2) encoding the chains of type I collagen. Recently, dysregulation of hydroxylation of a single proline residue at position 986 of both the triple-helical domains of type I collagen α1(I) and type II collagen α1(II) chains has been implicated in the pathogenesis of recessive forms of OI. Two proteins, CRTAP, or cartilage-associated protein, and prolyl-3-hydroxylase-1 (P3H1, encoded by the LEPRE1 gene) form a complex that performs the hydroxylation and brings the prolyl cis-trans isomerase cyclophilin-B (CYPB) to the unfolded collagen. In our screen of 78 subjects diagnosed with OI type II or III, we identified three probands with mutations in CRTAP and sixteen with mutations in LEPRE1. The latter group includes a mutation in patients from the Irish Traveller population, a genetically isolated community with increased incidence of OI. The clinical features resulting from CRTAP or LEPRE1 loss of function mutations were difficult to distinguish at birth. Infants in both groups had multiple fractures, decreased bone modeling (affecting especially the femurs), and extremely low bone mineral density. Interestingly, “popcorn” epiphyses may reflect underlying cartilaginous and bone dysplasia in this form of OI. These results expand the range of CRTAP/LEPRE1 mutations that result in recessive OI and emphasize the importance of distinguishing recurrence of severe OI of recessive inheritance from those that result from parental germline mosaicism for COL1A1 or COL1A2 mutations.

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The goal of our laboratory is to understand the mechanisms that underlie human pituitary development and function and to apply that knowledge to the prevention and treatment of pituitary diseases. The anterior pituitary gland secretes polypeptide hormones that are critical for many aspects of development and physiology, including growth, reproduction, the stress response, metabolic homeostasis, and lactation. Diseases involving pituitary dysfunction therefore can involve severe syndromes affecting many tissues and systems. During embryogenesis, the actions of multiple regulatory transcription factors, such as the LHX3 homeodomain protein, govern the establishment of the hormone-secreting pituitary cell types. This laboratory and others have described that mutations in the LHX3 gene (and in the related LHX4 gene) cause combined pituitary hormone deficiency diseases, and other symptoms such as nervous system problems, in children. The biochemical mechanism by which LHX3/4-class proteins exert their functions is poorly understood. We have, however, recently determined that mutations affecting distinct parts of the LHX3 protein are correlated with specific disease outcomes: for example, mutations only compromising the carboxyl terminus are associated with a more restricted disease involving pituitary hormone deficiency but not nervous system problems. Ongoing experiments are investigating the mechanism of LHX3-mediated transcription by characterizing protein complexes that interact with the domains of LHX3 that are critical for pituitary gene regulation. To further understand the novel forms of LHX3-associated diseases involving specific symptoms, we are generating mouse models carrying equivalent mutations, allowing molecular and cellular characterization of the disease symptoms and progression, an approach that is not possible in the patients. In addition, we are analyzing the mechanisms that regulate transcription of the human LHX3 gene using transgenic animal approaches. In collaboration with clinical endocrinologists in Indiana and elsewhere, the non-coding gene regulatory regions identified in these experiments are providing candidate sequences for tests of involvement in pediatric pituitary diseases of unknown etiology.
Left-right patterning and cardiac morphogenesis in zebrafish*

My laboratory studies organ morphogenesis and patterning the zebrafish. We are particularly interested in the genes and morphological events that govern patterning along the left-right (LR) axis. While asymmetric Nodal signaling plays important roles in directing the placement of asymmetric organs, it is unclear how Nodal signaling influences specific events in morphogenesis. There are two distinct cardiac asymmetries in zebrafish: cardiac jogging and cardiac looping. Our analysis of asymmetric gene expression in the heart suggested jogging involved a rotational migration event and an axis conversion event. We have confirmed that Nodal signaling influences the direction of migration within the cardiac cone leading to a conversion of cells from the left to the dorsal side of the heart tube. Using fate mapping experiments we have confirmed these results and determined that a second rotation of the heart occurs just prior to looping which re-establishes the original left-right position of the cells. This rotation is independent of Nodal signaling. Using mutants that predictably alter the sidedness of nodal, we have confirmed that Nodal signaling directs the axis conversion during jogging, but not prior to looping. These recent studies have provided intriguing insights into the role of Nodal in directing axis conversions during heart morphogenesis. Furthermore, our results suggest cells derived from the left and right lateral plate mesoderm have some side specific information that is independent of Nodal signaling.

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WEDNESDAY ABSTRACTS
An Update on the National Birth Defects Prevention Study

The National Birth Defects Prevention Study is a collaborative study to identify genetic and environmental causes of major birth defects. The eight currently funded sites are: 1) Arkansas, 2) California, 3) Iowa, 4) Massachusetts, 5) New York, 6) North Carolina, 7) Texas, and 8) Utah. CDC participates as the ninth site of the study with the project site in Atlanta, Georgia. The study: 1) identifies infants with major birth defects using population-based surveillance systems and clinician review of all cases, 2) interviews mothers about their medical history, environmental exposures, and lifestyle, 3) collects DNA from infants and parents to study gene-environment interactions, 4) establishes a specimen bank to store biologic samples for future study, and 5) provides a continuing source of information on potential causes of birth defects and serves as a mechanism for identifying new substances in our environment that are harmful to developing babies.

As of April 2008, we had completed nearly 30,000 maternal interviews and collected and processed nearly 14,000 infant DNA samples. CDC established a centralized laboratory in 2003 to improve the quality and consistency of sample processing. Combined with the implementation of a new collection brush, these improvements have significantly increased the quantity of DNA available for analyses, particularly for infants.

Over the past 12 months, the study has published key findings on risk factors for birth defects. Some examples include: 1) Use of selective serotonin-reuptake inhibitors in pregnancy and the risk of birth defects (Alwan et al., NEJM, 2007), 2) Seeking causes: a clinical classification of cardiovascular malformations for use in etiologic studies (Botto et al., BDRA, 2007), 3) Maternal Smoking and congenital heart defects (Malik et al., Pediatrics, 2008), 4) Maternal thyroid disease as a risk factor for craniosynostosis (Rasmussen et al., Obstet Gynecol, 2007), and 5) Prepregnancy obesity as a risk factor for structural birth defects (Waller et al., Arch Pediatr Adolesc Med, 2007).
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Update on “Gene Mutation and Rescue in Human Diaphragmatic Hernia”*

To identify genetic causes of congenital diaphragmatic hernia (CDH), we continue to recruit patients into a large study focused solely on patients with CDH. To date, 334 patients and 540 of their parents participate in this study, 75% recruited from our Boston sites and the remainder recruited from around the world. Clinical information is collected on each patient for phenotyping into Isolated or Complex CDH. A DNA sample is obtained, which in ~200 cases is extracted from a permanent cell line. Several approaches for gene discovery are being used:

1) array Comparative Genomic Hybridization- 52 patients comprising a mix of Complex and Isolated CDH patients were first interrogated using the 1 Mb Spectral array. No abnormalities were found in Isolated CDH cases, but 3 previously unsuspected abnormalities were found in Complex CDH cases. Currently, a total of 50 Complex CDH cases are being interrogated for microdeletions, microduplications, and copy number variations (CNVs) using the Agilent 244K oligonucleotide array platform. Initial results demonstrate a total of 68 previously unreported copy number changes, 47 of which include coding sequence. Parental genotyping and verification of the variants by quantitative PCR is underway.

2) Gene identification in single gene disorders- Several multiplex kindreds, whose phenotype includes CDH as part of apparent monogenic syndromes, have been recruited. Homozygosity mapping in a large consanguineous family with several Donnai-Barrow syndrome (DBS) children led to the discovery that mutations in the LRP2 gene, encoding megalin, cause this disorder. Work on delineating the core DBS phenotype and developing care guidelines will be presented.

3) Candidate gene sequencing- A micro-deletion encompassing chromosome 1q42, detected using the Spectral array platform discussed above, revealed an attractive candidate gene, DISP1, mapping to this locus. Initially sequenced in 24 complex CDH patients, we identified a de novo mosaic mis-sense “mutation” in one case. High throughput sequencing of DISP1, and several genes of interest mapping to other chromosomes including LRP2, MEF2A, RAP, HLXI, and STRA6 is underway on 196 CDH study patients.

4) Unbiased identification of functional associations between genes in CNV regions- To investigate the hypothesis that CDH-related genes interact as part of one or several pathways, we created a human protein protein interaction (ppi) network spanning a set of 54 proteins involved in CDH or related phenotypes in humans or mice. This network is significantly enriched for interactions (P=2.2e-6) between this set of proteins compared to randomly generated networks, providing support of our hypothesis. To extend this analysis further, we identified proteins mapping to the CNV regions seen on the Agilent 244K array, and investigated if they could be placed in one or several significant ppi subnetworks which would suggest shared function. The preliminary analysis identifies 5 significant subnetworks spanning 15 proteins, including two already known CDH-related proteins (GATA4 and COUP TF2), hereby providing proof of principle. These initial results are encouraging us to explore whether novel candidates identified by this approach connect to the 54 CHD-related proteins.

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Identifying Genes for Congenital Diaphragmatic Hernia*

Congenital diaphragmatic hernia (CDH) is a relatively common sporadic birth defect, with an incidence of approximately 1:3,000 live births. Although CDH can occur in isolation, approximately 50% of cases occur with additional anomalies. The sporadic nature of this defect makes linkage-based approaches to gene identification impractical. In an alternative approach, we are using a positional candidate strategy based on chromosomal data to localize and identify genes that cause or predispose to the development of CDH. By reviewing published case reports, we have identified 19 chromosomal regions that are recurrently deleted or duplicated in CDH. These regions are distributed in a non-random pattern that is distinct from that seen in other birth defects such as esophageal atresia/tracheoesophageal fistula. We hypothesize that each of these regions harbors a gene, or genes, related to CDH. To identify new CDH-related regions—and refine those previously reported—we are screening for cryptic deletions and duplications in affected individuals using high density genome-wide array comparative genome hybridization. Changes which have not been identified previously in normal individuals are confirmed and their inheritance pattern is determined by real-time quantitative PCR. Using this combined approach we have identified over 50 rare genomic variants in 34 patients with CDH including an approximately 700kb de novo deletion on chromosome 16p and de novo duplications involving regions of chromosome 11q and 13q.

In a second approach we are using mouse models to study the interactions between various CDH-related genes. We have recently discovered that mutations in the extracellular matrix protein Frem1 can cause retrosternal sac hernias, lung segmentation defects and renal agenesis. These defects are similar to those seen in mice with mutations in Slit3 and Gata4. We are presently conducting in vivo experiments to determine if decreased expression of Slit3 and Gata4 exacerbate portions of the Frem1 phenotype.

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Microarray Studies in a Male with Congenital Diaphragmatic Hernia and Anophthalmia reveal a Novel 18q22.1 Deletion

Congenital diaphragmatic hernia (CDH) is a common birth defect with a high mortality and morbidity. Identification of the causative genes for CDH would improve genetic counseling for this malformation and an improved understanding of diaphragm formation may lead to new therapies to enhance diaphragm function in neuromuscular diseases. We have performed array studies in 16 patients with CDH and additional anomalies using the using the Affymetrix GeneChip®100K Set. Chip data was analyzed using the methodology of Barross et al. (BMC Bioinformatics 2007;8:368). In a male patient with CDH and anophthalmia, array data showed copy number changes consistent with a novel 2.7 Mb deletion at chromosome 18q22.1. This entire deletion has not been noted as a copy number variant (http://projects.tcag.ca/variation/). Parental studies showed that the deletion was maternally inherited; however, as there is a maternal aunt with anophthalmia, variable expressivity or non-penetration is possible.

The 18q22.1 deletion was mapped using FISH and contains only four known genes. In-situ hybridization with murine embryo sections showed expression of two of the deleted genes in the diaphragm and eye. The first gene, Dsel, was weakly expressed in the murine diaphragm and eye at E13.5. DSEL converts D-glucuronic acid to L-iduronic acid in dermatan sulfate biosynthesis and decorin, the main dermatan sulfate proteoglycan, is synthesized in myogenic cells and promotes proliferation in C2C12 myoblasts. We therefore re-sequenced DSEL in 120 CDH patients. We found c.G42A, predicting p.M14I in one patient with antral CDH. This alteration was not present in 200 control chromosomes and the amino acid residue is highly conserved. We also found an increased frequency of the SNP c.A827G predicting p.N276S in patients (allele frequency A = 0.94 G =0.06) compared to controls (allele frequency A = 0.98 G = 0.02; p= 0.03). We are collaborating with Drs Maccarana and Maelstrom (University of Lund) who are studying DSE and DSEL to perform functional studies to determine the significance of these variants.

The second gene, Txndc10, showed moderate expression in the murine eye from E11.5 onwards. TXNDC10 is a thioredoxin that catalyzes the formation of disulfide bonds. As thioredoxins are vital to normal lens formation, we re-sequenced this gene in 66 anophthalmia/microphthalmia patients. We found one alteration, c.G260A, predicting p.R39Q, that is located in the thioredoxin-like domain of the protein and thus highly conserved. We are planning morpholino studies with zebrafish to establish if this gene is important in eye formation (collaborator Dr Baier, UCSF).

Our work emphasizes the difficulties with gene identification in sporadic birth defects – there is significant genetic heterogeneity for both CDH and anophthalmia, and we have found few sequence variants in our candidate genes. Functional and/or animal studies are also necessary for proof of gene involvement. We are therefore developing an approach that uses siRNA to knock down gene expression in primary myoblast or C2C12 cells for functional studies of cell proliferation and migration.