34th Annual Meeting of the Society of Craniofacial Genetics and Developmental Biology

Presents
Clinical Craniofacial Dysmorphologies
with featured speakers

Roderick R. McInnes, M.D., McGill
Debra Krakow, M.D., UCLA
Marilyn Jones, M.D., UCSD
Monzur Murshed, Ph.D., McGill
Brian K. Hall, Ph.D., Dalhousie

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### Clinical Craniofacial Dysmorphologies

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**Poster Abstract Information**

The first author is the presenting author. The presenter will share a 4X8 foot board with one other author. We recommend a poster size of 2’10” (86.36 cm) wide by 3’8” (111.8 cm) high. We invite the author to put up their poster in the morning.
Roderick R. McInnes, MD, PhD, was recently appointed to his new position as the Alva Chair in Human Genetics at McGill University, and Director of the Lady Davis Research Institute at the Jewish General Hospital of McGill. From 2000-2010, he was the inaugural Scientific Director of the Institute of Genetics of the Canadian Institutes of Health Research (CIHR) and in 2010, he served as President of the American Society of Human Genetics.

Prior to joining McGill, Dr. McInnes was a University Professor of the University of Toronto, the Anne and Max Tanenbaum Chair in Molecular Medicine at the Hospital for Sick Children, Professor of Pediatrics and of Molecular and Medical Genetics, and an International Research Scholar of the Howard Hughes Medical Institute (HHMI). He is a co-author of the 5th, 6th, and 7th editions of Thompson and Thompson’s *Genetics in Medicine*.

Dr. McInnes has made many important contributions to the understanding of the molecular basis of retinal and eye development, and to the identification of genes and processes associated with inherited retinal degenerations. Recently, he and collaborators identified an important protein, Neto1, required for learning and memory, and established that it is possible to correct an inherited learning defect in mice with a drug, a finding with important implications for human learning disability.

Among other honors, Dr. McInnes is a Fellow of the Royal Society of Canada and the Canadian Academy of Health Sciences. He was the recipient of the Samuel Rosenthal Award from the Rosenthal Foundation of Cleveland in 2002, and an honorary Doctor of Laws from Dalhousie University in 2007. Dr. McInnes was appointed to the Order of Ontario in 2008, and became a member of the Order of Canada in 2009.
Dr. Marilyn Jones is Professor of Clinical Pediatrics at the University of California, San Diego. She is a graduate of Wellesley College and the Columbia University College of Physicians and Surgeons. She received her pediatrics training at the University of California, San Diego, where she also completed a fellowship in dysmorphology with Kenneth Lyons Jones. Since 1979 she has been director of Dysmorphology and Genetics at the Rady Children's Hospital, San Diego. In 1980 she became director of the Cleft Palate and Craniofacial Treatment Programs and also the medical director of the Bernardy Center for Medically Fragile Children. She has been active in a number of professional organizations, serving on the council then as president of the Western Society for Pediatric Research; the council and then president of the American Cleft Palate and Craniofacial Association; and the board of directors and then president of the American College of Medical Genetics. She has an active interest in the causes of birth defects particularly with reference to cleft and craniofacial disorders.
Dr. Deborah Krakow is Professor of Orthopaedic Surgery, Human Genetics and Obstetrics and Gynecology, at the David Geffen School of Medicine at UCLA in Los Angeles, California. She is a member of the International Skeletal Dysplasia Registry, a NIH program project that studies the natural history, treatment, and underlying mechanisms that lead to the skeletal dysplasias. Dr. Krakow has identified the molecular basis of numerous disorders including the “Filamin B-opathies,” multiple synostosis syndrome, TRPV4 disorders and short-rib polydactyly syndrome. She has a long-standing interest in using prenatal ultrasound to define the defects in Mendelian disorders.
Dr. Monzur Murshed is a member of the Mineralized Tissues and Extracellular Matrix Biology Group, Faculty of Dentistry, McGill University. The Murshed laboratory primarily focuses on the genetic regulation of tissue mineralization. Bones and teeth are the tissues where physiologic mineral depositions take place. These mineralized tissues serve important biomechanical functions and also act as a reservoir for essential mineral ions required in vital cellular activities. Soft tissue mineralization, on the other hand, is pathologic, which often leads to debilitating conditions. Mineral deposition in the arterial walls can be a risk factor for many cardiovascular diseases, while such deposits in the joints can cause osteoarthritis a common chronic joint condition of the elderly in Canada. Our working hypothesis suggests that mineral deposition in a tissue depends on the availability of two key mineral ions calcium and inorganic phosphate, the presence of a suitable mineral scaffolding protein matrix and the extracellular levels of inhibitors that prevent mineral crystal precipitation and growth. We use the power of modern mouse genetics in combination with a range of molecular and cell biology techniques to uncover the novel genetic regulators of tissue mineralization and their mechanisms of action. Revealing these regulators should eventually help identify new therapeutic targets and improve the management of complications associated with abnormal tissue mineralization.
Brian K. Hall is University Research Professor Emeritus at Dalhousie University in Halifax, NS. He has spent his career in the study of the embryonic development and evolution of the skeleton and skeletal tissues, especially neural crest-derived tissues of the craniofacial skeleton and biomechanical effects on cartilage development. In recent years he has paid particular attention to the role of cell condensations in skeletal development, especially in the mammalian mandible — the topic of his presentation at this meeting. The author of numerous papers, reviews, books and textbooks and known for his ability to synthesize and integrate, he is regarded as one of the founders of the field of “Evolutionary Developmental Biology” or ‘evo-devo.’ A Fellow of the Royal Society of Canada and a Foreign Honorary Member of the American Academy of Arts & Sciences, he received the International Craniofacial Biology Distinguished Scientist Award in 1996, the Alexander Kowalevsky Medal in 2001, and the Killam Prize from the Canada Council for the Arts in 2005. The Hall Award for the best student platform paper presented in the Comparative Morphology and Development Division of the annual meeting of the Canadian Society of Zoologists was established in 2006.
Inherited retinal disorders: hope for treatment

Roderick R. McInnes, MD PhD
Lady Davis Institute, McGill University

Inherited retinal diseases can be broadly classified into developmental defects and retinal degenerations. I will give a brief overview of these conditions, and focus in particular on our current understanding of the mechanisms that underlie photoreceptor death in the inherited degenerations. In our work on these diseases, we are addressing two fundamental questions: First, why do the neurons die? And second, how is it that they can function perfectly normally for decades, yet still be at risk of death? Are they sick? What are the biochemical changes that result from the mutation, and that eventually kill the cells? Some insight into these difficult questions has been obtained by many groups over the past decade. I will also review exciting progress in the treatment of inherited retinal disease, both cell replacement therapy and gene therapy. In Phase 1 clinical trials, gene therapy appears to have been effective in partially correcting the blindness of at least one retinal degeneration, and cell replacement therapy in mouse models of retinal degeneration is very promising. Finally, I will report new findings on the role of one transcription factor gene, Prdm8, in retinal development. Loss of function of this gene leads to a virtual absence of bipolar cells, the major interneurons in the retina, and also to fascinating neuromuscular abnormalities.

References

What can One Learn from Fetal Facies: Is it a Clue to Diagnosis?

Deborah Krakow, MD
Department of Orthopaedic Surgery, Human Genetics and Obstetrics and Gynecology

Improvements in prenatal fetal ultrasound based on technological advancements have yielded superior images of many organ systems throughout gestational ages. This is especially true of the fetal facies. Ultrasound can evaluate both the bony structures as well as the soft tissue contours. Absolute measurements of the orbital diameters, philtrum, and mandible can give definite evidence for hypo- and hypertelorism, abnormal philtrums, and micrognathia. The recognition of well-described craniofacial disorders can be translated into the fetal period, as soon as the early second trimester. Abnormal craniofacial finding can be readily appreciated in the skeletal dysplasia group of disorders, craniosynostosis group of disorders, and cleft/lip palate syndromes. Determining the constellation of abnormal facial findings can help direct the prenatal geneticists toward differential diagnoses, including recognition of novel disorders. These diagnoses can then be refined based on abnormalities in other organ systems and well as using molecular diagnostics to help identify the causative mechanisms.
Genetic Regulation of Bone Extracellular Matrix Mineralization

Dr. Monzur Murshed
Faculty of Dentistry. McGill University

Mineralization of vertebrate bone and tooth extracellular matrix (ECM) is a physiologic process. In contrast, soft tissue mineralization, also known as ectopic mineralization, is a pathologic condition. Initiation of ECM mineralization requires a scaffold of fibrillar proteins such as collagen or elastin within which critically sized nuclei of salts of calcium and inorganic phosphate (Pi) precipitate and become stable. These precipitates later grow and mature into hydroxyapatite crystals. Interestingly, although suitable scaffolding proteins are present in many soft tissues including skin and arteries, normally, ECMs in these tissues do not mineralize. There are two possibilities that may explain this phenomenon. Firstly, it is possible that an activator of mineralization is missing in these soft tissues, and secondly, that soft tissue mineralization is actively prevented by the presence of anti-mineralization (inhibitor) molecules. Several key studies now indicate that the latter scenario is probably most likely the case. One of the latest additions to the growing list of mineralization regulators is Sphingomyelin phosphodiesterase 3 (Smpd3). Smpd3 encodes a neutral sphingomyelinase that cleaves sphingomyelin to generate bioactive lipid metabolites—ceramide and phosphocholine. A deletion mutation called fragilitas ossium (fro) in the murine Smpd3 gene leads to severe skeletal dysplasia and perinatal death. We observed an impaired apoptosis of the hypertrophic chondrocytes and severely under-mineralized cortical bones in E15.5 fro/fro embryos. Our genetic experiments suggest a tissue specific role for Smpd3 in skeletal mineralization. Furthermore, we show that feeding fro/fro mice a choline-rich diet can significantly reduce the bone mineralization defects. These data identifies SMPD3 as a novel regulator of skeletal development and mineralization.
Mammalian mandibular modules: 20 years since the "Atchley-Hall" Model

Brian K. Hall1
1 Department of Biology, Dalhousie University, Halifax NS Canada B3H 4J1

It is 20 years since Atchley and Hall published a model for the development and evolution of complex morphological structures using the mammalian mandible (dentary) as the exemplar anatomical structure (1999, Biol. Rev. 66: 101-157). Included in the model was the development of the concept of modularity of morphology, modules consisting of aggregations (condensations) of cells that are the primary resource for the development of individual bones or cartilages. In this first of a series of presentations/papers to review the model, I examine our current understanding of cellular modules of the murine dentary. The 1991 model postulated that the dentary arose from six cell condensations of neural-crest-derived cells. Four were skeletogenic forming the ramus and the three processes of the dentary. Two were odontogenic, forming the incisor and molar teeth and associated alveolar bone. Subsequent studies reveal that a single skeletogenic unit forms the bone of the ramus and the angular, condylar and coronoid processes. In mice the distal cartilages on these processes are secondary, arising from the periosteum. In rats and humans these cartilages are sesamoids arising in separate condensations outside the dentary. Thus, the single osteo-chondrogenic condensation in mice is represented in rats and humans by four cell populations; one osteogenic and three sesamoid (chondrogenic) condensations. The significance of these differences for our understanding of the cellular and molecular mechanism underlying mandibular development and for the application of studies from other mammalian species to human craniofacial development will be documented and discussed.

Supported by NSERC of Canada (A5056).
GWAS follow-up mutation screen and expression analysis implicate ARHGAP29 as a novel candidate gene for nonsyndromic cleft lip/palate

Elizabeth J. Leslie,1 M. Adela Mansilla,1 Leah C. Biggs,1 Kristi Schuette,1 Steve Bullard,2 Tian-Xiao Zhang,3 Margaret Cooper,4 Martine Dunnwald,1 Andrew C. Lidral,2 Mary L. Marazita,4 Terri H. Beaty,3 Jeffrey C. Murray1

1- Department of Pediatrics, University of Iowa, Iowa City, Iowa, USA.
2- Department of Orthodontics, University of Iowa, Iowa City, Iowa, USA
3- Department of Epidemiology, School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA
4- Center for Craniofacial and Dental Genetics, Department of Oral Biology, School of Dental Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Nonsyndromic cleft lip and/or palate (NSCL/P) is a common birth defect with complex etiology. Genome wide association studies have successfully identified novel loci associated with NSCL/P including one near the ABCA4 gene, mutations in which cause several retinal disorders. Neither expression analysis nor mutation screening support a role for ABCA4 in the etiology of NSCL/P, so we investigated the adjacent gene, ARHGAP29, encoding Rho GTPase activating protein 29. ARHGAP29 has preferential activity toward RhoA, which has many functions related to cellular shape, movement, and proliferation, all critical for craniofacial development. Expression analysis using a mouse demonstrated that Arhgap29 is present in the epithelium and mesenchyme of the medial and lateral nasal processes and the mandibular processes at E10.5, and the oral and medial edge epithelia and palatal mesenchyme at E14.5. Sequencing of ARHGAP29 in 962 individuals with NSCL/P and 972 unrelated controls from the Philippines and the U.S. revealed one nonsense, one frameshift, and fourteen missense variants, which are overrepresented in cases (p=0.03). We tested the most associated SNP (rs560426) near ABCA4 and ARHGAP29 for genetic interaction with other candidate genes, identifying a possible interaction with IRF6 (rs2235371) (p=0.04). This interaction is supported by reduced expression of Arhgap29 in the oral epithelium of an Irf6-null mouse, suggesting a novel pathway for clefting involving the transcription factor IRF6 interacting with the Rho pathway via ARHGAP29. The combination of genome wide association, rare coding sequence variants, craniofacial expression, and interactions with a known clefting gene support a role for ARHGAP29 in NSCL/P.
Dental pulp regeneration via amplification of an endogenous Wnt signal.

D.J. Hunter (Department of Surgery, Division of Plastic and Reconstructive Surgery, Stanford School of Medicine, Stanford, CA 94305, USA.) S. Suzuki (Department of Endodontics and Periodontology, Hiroshima University Faculty of Dentistry, 1-2-3 Kasumi, Minami-ku, Hiroshima, Japan.) and J.A. Helms (Department of Surgery, Division of Plastic and Reconstructive Surgery, Stanford School of Medicine, Stanford, CA 94305, USA.)

Adult tissues contain stem cells, which can be activated in order to repair damaged tissue. Dental pulp is one such tissue but the extent of activation following pulp injury is unknown. We hypothesized that the response of dental pulp stem cells could be amplified by transient exposure to an exogenous Wnt stimulus and that the progeny could differentiate into dentin-producing odontoblasts, aiding injury repair. We tested this using Axin2LacZ/+ reporter mice, which allow for observation of endogenous Wnt signaling. We first demonstrated that dentin-producing odontoblasts respond to endogenous Wnt signals and perturbations disrupt normal production. These results indicate Wnt signals are part of the normal maintenance of odontoblast function. We then employed a genetic strategy to amplify the Wnt response to injury. To do this we used Axin2LacZ/LacZ mice, where loss of both copies of the negative Wnt regulator, Axin2, leads to an enhanced Wnt response (Minear et al., 2010). We generated molar pulp injuries and found pulpal cell death in Axin2LacZ/+ whereas in Axin2LacZ/LacZ mice there was a robust response: the injury site was filled with a mineralized tissue that obturated the dentin defect. This strongly suggests that enhancing the endogenous Wnt signal may induce dental pulp stem cells to generate reparative dentin in response to injury. We intend to test this possibility by locally delivering a form of Wnt3a protein (Morrell et al., 2009). Collectively, these experiments suggest that we can amplify the body’s natural response to injury: leading to more effective repair of dental pulp injuries.
Autosomal dominant multiple natal teeth with selective tooth agenesis

John M. Graham, Jr.,1 Nancy Kramer,1 Vincent Funari,1 Ophir Klein,2 Kerstin Seidel,2 Piranit Kantaputra,3 Kent D. Taylor1

1 Medical Genetics Institute, Cedars Sinai Medical Center, Los Angeles, CA
2 Dept Orofacial Sciences, University of California, San Francisco, CA
3 Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand

We report a 5-generation family with the combination of autosomal dominant multiple natal teeth with subsequent selective tooth agenesis not associated with any non-dental features. Natal teeth are usually a sporadic isolated finding in an otherwise normal infant. Familial occurrence is rare but has been reported to be autosomal dominant. Autosomal dominant agenesis of teeth can be caused by mutations in the homeobox gene MSX1. Other families with autosomal dominant oligodontia have mutations in PAX9, and selective agenesis of only the permanent teeth was linked to 10q11.2-q21. A family with isolated X-linked selective tooth agenesis resulted from mutations EDA. Mutations in WNT10A have been associated with isolated hypodontia. In the family we report, natal teeth with hypodontia was not associated with ectodermal dysplasia or any other problem. DNA from 28 family members was analyzed on the Illumina OMNI-express chip using 733,120 SNPs and mapped to an approximately 2Mb segment on chromosome 1q36.11 with LOD score 2.97 at 23.8 Mb to 25.8 MB (GRCh37/hg19; MERLIN). By dividing the pedigree into three 3-generation families, a region of association was found located between LOC284632 and GRHL3 (parenTDT, p=0.005 for rs11249039, rs11249045, or rs7526505). GRHL3 is a gene expressed exclusively in surface ectoderm in drosophila, where it plays an essential role in cuticle formation. Expression of the murine Grhl3 gene is also found in ectodermally derived tissues including the oral epithelium. We speculate that variation in the regulation of this gene might play a role in the phenotype we describe in this abstract.
Is the craniofacial phenotype sufficient to characterize FGFR-related craniosynostosis syndromes?

Yann Heuzé1, Neus Martínez-Abadías1, Jennifer M Stella1, Federico Di Rocco2, Corinne Collet3, Gemma García Fructuoso4, Mariana Alamar4, Lun-Jou Lo5, Simeon A Boyadjiev6, and Joan T Richtsmeier1 (1Department of Anthropology, Pennsylvania State University, University Park, PA, USA / 2Craniofacial Surgery Unit, Department of Pediatric Neurosurgery, Hôpital Necker–Enfants Malades, Université Paris V, Paris, France / 3Laboratoire de Biochimie et de Biologie Moléculaire, INSERM U606, Paris, France / 4Servei de Neurocirurgia, Hospital Sant Joan de Déu, Barcelona, Spain / 5Department of Plastic and Reconstructive Surgery, Chang Gung Memorial Hospital, Chang Gung University, Taoyuan, Taiwan / 6Section of Genetics, Department of Pediatrics, University of California Davis, Sacramento, California, USA)

More than 180 craniosynostosis syndromes (CS) have been described with an overall prevalence of 1 per 10,000 live births. The more common CS are associated with mutations in fibroblast growth factor receptors (FGFRs). These “FGFR-related CS” show the characteristic premature fusion of one or several cranial sutures along with additional craniofacial, neural, limb, heart, lung, and/or skin anomalies. Despite the potentially high number of causative mutations for some of these FGFR-related CS, clinical diagnoses are quite reliable. However, our morphometric analysis based on landmarks collected from skull CT images of patients with Apert (n=19), Crouzon (n=9), Pfeiffer (n=5) and Muenke (n=4) syndromes along with those of unaffected children (n=20) shows that these diagnostic categories are more difficult to establish when skull shape is the only trait considered. Indeed, we observe substantial overlap between craniofacial phenotypes, particularly of Apert, Pfeiffer and Muenke syndromes. The craniofacial phenotype as characterized by CT data is not sufficient to characterize FGFR-related CS. The cases that are most different from the unaffected individuals are the syndromic cases with bicoronal craniosynostosis. When syndromic cases displaying bicoronal craniosynostosis (n=17) are compared with unaffected individuals (n=20) and with children presenting with nonsyndromic bicoronal craniosynostosis (n=14), our data provide a clear separation between craniofacial phenotypes of syndromic and nonsyndromic cases. The syndromic cases with bicoronal craniosynostosis display frontal bossing and severe midfacial hypoplasia. These last results indicate that in the case of bicoronal craniosynostosis the FGFR-related causative mutation and/or the molecular pathway affected by this mutation generates additional cranial dysmorphologies.
We recently proposed a two-hit model to explain the phenotypic variability associated with a 520-kbp microdeletion on chromosome 16p12.1, wherein, the microdeletion both predisposes to neuropsychiatric phenotypes as a single event and exacerbates neurodevelopmental phenotypes in association with other large (>500 kbp) copy number variants (CNVs). We extended our model to include 74 genomic disorders and examined CNV data from 32,587 cases with intellectual disability and congenital malformation for the presence of two large CNVs compared to 8,329 controls. Of the 2,374 cases with a known genomic disorder, 274 (11.5%) cases carried another CNV >500 kbp and 454 carried another CNV >150 kbp elsewhere in the genome. For 24/134 (17.9%) of these two-hit carriers, the second CNV was also associated with a genomic disorder. While the frequency of second hits was higher in CNVs associated with variable expressivity such as del15q13.3, del16p11.2, dup16p13.11, del16p12.1, and del and dup1q21.1, we found a positive correlation (Spearman correlation, r= 0.6, P=7.1×10^{-4}) between the proportion of inherited cases and the prevalence of the second hit. Analysis of parental DNA showed a combination of inherited and de novo events contributing to the occurrence of two hits in the probands. Pathway analysis of genes within the second hit CNVs showed disruption of genes involved in cellular signaling, neurological, and developmental functions. Our data provide strong support for the two-hit model to explain variable expressivity in genomic disorders and, overall, presents an oligogenic basis for the study of complex diseases.
A novel role for neutral sphingomyelinase 2 in bone mineralization

Zohreh Khavandgar1, Jingjing Li2 and Monzur Murshed1,2- 1Faculty of Dentistry and 2Department of Medicine, McGill University, Montreal, Quebec, Canada.

Mineralization of vertebrate bone and tooth extracellular matrix (ECM) is a physiologic process. These mineralized ECMs are essential for normal embryonic development and survival of the vertebrate animals. Two mineral ions, inorganic phosphate (Pi) and calcium, promote hydroxyapatite crystal growth within the newly synthesized collagen fibrils in the skeletal tissues. Another key determinant of ECM mineralization is the level of mineralization inhibitors in the tissue microenvironment. In skeletal hard tissues, an ectopic enzyme, tissue non-specific alkaline phosphatase (ALPL), cleaves a potent mineralization inhibitor inorganic pyrophosphate (PPi). We showed earlier that the unique co-expression of ALPL and Type I collagen in bone explains, at least in part, the tissue specificity of physiologic mineralization. New experimental models however suggest that there might be additional mediators regulating this process. A deletion mutation called fragilitas ossium (fro) in the murine sphingomyelin phosphodiesterase 3 (Smpd3) gene leads to an abnormal chondrocyte hypertrophy and poor bone ECM mineralization in the developing fro/fro embryos. In a recent in vivo study we showed that nSMase2 regulates bone mineralization locally in osteoblasts. Smpd3 encodes a neutral sphingomyelinase (nSMase2), which cleaves sphingomyelin to generate two bioactive lipid metabolites—ceramide and phosphocholine. Here we demonstrate a critical role for nSMase2 metabolite phosphocholine in in vitro mineral deposition by MC3T3-E1 pre-osteoblasts. Furthermore, we show that feeding fro/fro mice a choline-rich diet can significantly reduce the bone mineralization defects. Our data identifies nSMase2 as a novel regulator of skeletal development and mineralization.
A rare DNA variant in a cis-overlapping motif (COM) in an IRF6 enhancer element is associated with Van der Woude Syndrome

Walid D. Fakhouri1, Fedik Rahimov2, Huiqing Zhou3, Tianli Du1, Evelyn N. Kouwenhoven3, Hans van Bokhoven3,4, Jeffrey C. Murray2, Brian C. Schutte1,5

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Cleft lip and palate (CLP) is one of the most common birth defects in humans. Mutations in Interferon Regulatory Factor 6 (IRF6) cause Van der Woude syndrome (VWS), an autosomal dominant form of CLP, and contributes risk for isolated CLP, including a common DNA variant rs642961. Rs642961 is located in MCS9.7, a multi-species conserved sequence that is near the IRF6. The MCS9.7 element was shown to possess enhancer activity that mimicked the expression of endogenous Irf6. In order to identify possible etiologic DNA variants, we sequenced MCS9.7 in DNA samples obtained from individuals with VWS. We screened 48 DNA samples for which no disease-causing mutation was detected in IRF6 exons. We observed one new DNA variant that is an A insertion and is predicted to disrupt the DNA binding for both p63 and for bHLH transcription factors. We focused on four members of bHLH family whose expression pattern appeared to overlap with Irf6. Using a DNA binding assay, we observed that this DNA variant abrogated binding by p63 and reduced the binding affinity for the bHLH trans factors. In a transient transactivation assay, we observed strong enhancer activity by the MCS9.7 element. This activation was highly dependent on p63, and the activation was abrogated by the A insertion mutation. In conclusion, these data are consistent with the hypothesis that the rare DNA variant at the cis-overlapping motif in MCS9.7 is etiologic for VWS, and supports the rationale for additional mutation screening of the MCS9.7 enhancer element in patients with CLP.
A SYSTEMS BIOLOGY APPROACH TO CLEFT LIP AND PALATE

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The etiology of clefts may follow a multifactorial-threshold model. That model, however, fits poorly. Some clefts result from teratogenesis, while occasionally clefting is inherited as a simple Mendelian factor. Although human sibships are too small for testing, the recurrence frequency for clefts appears to approximate what would be expected in a two factor genetic cross, 6.25%. About 25% of the variance in clefting is attributable to known genetic pathways. It appears that CLPs result from alleles or teratogens which slow neural crest cell migration as the known pathways may. My group (Bowers et al 1987 & under submission) have found that CLPs are systematic disorders, not alterations of the head and face alone. Affected children sometimes have delayed ulna styloid formation, and frequently have reduced elbow breadths, with normal triceps skinfolds and arm circumferences. We found significantly negative standard deviation scores (Zs) for elbow breadth in a sample of 209 children, ages 2-18:11, divided by sex, age group, and whether the cleft was unilateral or bilateral. Average Zs ranging from -0.40 (p < 0.05) to -1.27 (p < 0.001). Only boys above age 7:7 with bilateral CLP had non-significant average Zs, and these too were negative. This suggests that one of the molecules contributing to the formation of both membranous and cartilaginous bone, such as the transcription factor RUNX2, may be involved. Here I start to trace the regulatory circuitry which may link Runx2 to the pathways with known involvement.
Alterations in postnatal craniofacial bone mineral density and volume in the Fgfr2Y394C/+ Beare-Stevenson cutis gyrata syndrome mouse model.

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A novel technique is used to quantify individual cranial bone volume and relative bone mineral density across the murine skull from micro-computed tomography images and, in doing so, highlights the association between low bone density, low bone volume, and craniosynostosis in the Fgfr2Y394C/+ mouse model of Beare-Stevenson cutis gyrata syndrome at P0 and at P8. While landmark based morphometric analysis indicates that the severity of dysmorphology in craniofacial form varies across the skull, the influence of the Fgfr2 Y394C mutation on rates of bone volume increase appear standard for all bones measured. These results suggest that this mutation influences bone cell activity across the skull, even at sites quite distant from the prematurely fused sutures that define craniosynostosis syndromes. The net volume reduction of high density material in some mutant bones suggests that osteoclast activity, in addition to that of osteoblast, is affected during this early postnatal period. This novel study provides important information on the effect of the Fgfr2 Y394C mutation on endochondral and intramembranous bone development across the skull, complementing the results of morphometric analyses, and providing the basis for hypotheses that can be tested with more in depth histological, molecular, and cellular studies.

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Assessment of oral microbiota of alcoholic rats using human bacteria DNA probes.

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Background: Molecular diagnostic methods using genetic material for bacterial identification and quantification are faster and more reliable than conventional methods. This study aimed to evaluate the capacity of whole-genomic DNA probes prepared from human oral bacteria to detect and quantify oral bacterial species of rats, and to assess the influence of alcohol consumption on the oral biofilm.

Materials and methods: 12 adult Wistar-Kyoto rats were exposed to 20% ethanol mixed with their daily diet for 30 days and 12 rats in the control group did not receive alcohol in their diet. Upon euthanasia, bacterial samples from the oral biofilm covering the animals’ teeth were collected using microbrushes. Bacteria identification and quantification was performed using a DNA-DNA checkerboard hybridization method with 33 probes prepared from human oral bacteria. Bacterial counts and mean proportions were compared using a Mann-Whitney U test with a significance level < 0.05.

Results: All target strains, except S. mutans and S. mitis, were detected in the control group. E. coli, P. aeruginosa, P. endodontalis and V. parvula were the only species detected in the alcohol-treated group. A significantly higher number of bacteria was noted in the control group compared to the alcohol-treated group (p=0.001). The mean proportion of E. coli was highest in both groups.

Conclusion: Whole-genomic DNA probes prepared from human oral bacteria can cross-react with rats oral bacterial strains, and alcohol consumption is associated with reduced bacterial diversity and numbers in the oral cavity of rats.
Craniosynostosis, the premature fusion of one or more cranial sutures, occurs in approximately 1 in 2,500 live births. Among various forms, midline sagittal nonsyndromic craniosynostosis represents the most prevalent type. However, the etiology of NSC is largely unknown. We used data from an ongoing, population-based case-control study to identify novel candidate genes of NSC. Diagnosis was confirmed by radiographic images. Family history data are being collected from mothers, and saliva samples are being collected from both index child and birth parents in case and unaffected control families. We evaluated 96 NSC cases and performed extensive candidate gene analysis by direct sequencing. We identified three novel variants among different patients. In case 1, we found a frameshift variant in FGFR1 isoform 6 (c.732_733insG) which was predicted to abolish the entire immunoglobin III domain. In case 2, a variant in TWIST1 (c.439C>G) was observed at the highly conserved loop domain and may affect the DNA binding ability; the mother who had jaw surgery was confirmed to harbor the same variant. In case 3, a variant in RAB23 (c.546A>C) was identified. These variants detected in our study were unique and did not occur in 116 alleles from healthy control children from the same population. Our data suggest that mutations in these candidate genes may contribute to NSC, although they would account for a small proportion of total cases, and add to the perception of craniosynostosis as a complex developmental anomaly under potential polygenic control.
Case-parent trio genome-wide association study identifies several candidate loci for nonsyndromic sagittal craniosynostosis

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Craniosynostosis is a common malformation in which one or more of the cranial sutures (metopic, coronal, sagittal and lambdoid) in an infant skull fuse prematurely. Sagittal craniosynostosis is the most common type, accounting for 40-58% of all cases, with a prevalence of ~1.9-2.3 per 10,000 live births. The International Craniosynostosis Consortium (genetics.ucdmc.ucdavis.edu/icc.cfm) has been established to identify genetic causes of nonsyndromic craniosynostosis and more than 720 families with at least one affected individual have been recruited. To identify genetic variants associated with nonsyndromic sagittal craniosynostosis, we performed genome-wide association analysis of 201 case-parent trios and 13 nuclear families with two affected siblings. A total of 662 individuals in 214 families were genotyped on the Illumina 1M Human Omni1-Quad array. Association between sagittal craniosynostosis and each SNP was measured using transmission disequilibrium testing (TDT), implemented by PLINK v1.0.7. The strongest association was with rs1884302 (p = 3.79 X 10^{-14}) in the flanking 3’ UTR of BMP2 on chromosome 20. BMP2 belongs to the transforming growth factor-beta (TGFB) gene family and is involved in bone and cartilage formation. Genome-wide significant (p ≤ 5 X 10^{-8}) associations were also detected for SNPs intronic to BBS9 (rs1420154, p=3.51 X 10^{-13}) on chromosome 7, which is thought to be involved in parathyroid hormone action in bones. Additionally, we have strong but not genome-wide significant signals on DLG1 (rs12152266, p=1.44 x 10^{-7}), RPS12 (rs9493468, p=2.8 X 10^{-7}) and LOC643631 (rs1948330, p=4.78 X 10^{-7}). Loss of heterozygosity
mapping identified several chromosomal regions which are undergoing further analysis.
Decreased expression of Wnt9b, Wnt3, Adcy2, and Ube2q in the face of CL/Fr mouse embryos at E11.5 based on microarray analysis.

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The CL/Fr mouse demonstrates heritable bilateral and unilateral cleft lip and palate (CLP) at a rate of approximately 35%, generally above the background “A” strain mouse. Using classical mouse breeding strategies, it has been suggested that at least two disease loci, clf1 and clf2, are involved in the defect and candidate genes have been identified. Additionally, gene-targeting analyses strongly suggest that Wnt9b contributes to CLP in the “A” strain mice. The aim of this study was to test the expression of clf1 and clf2 candidate genes in the facial prominences of CL/Fr embryos, utilizing microarray analysis. Medial nasal, lateral nasal, and maxillary prominences of phenotypically normal as well as cleft E11.5 CL/Fr mice were dissected and RNA was extracted using standard techniques for Agilent-microarray protocol. Results indicate that expression of the clf1 candidate genes, Wnt9b, Wnt3, and clf2 candidate genes Adcy2, and Ube2q1 are significantly reduced (-3.11, -1.50, -2.22, and -1.83 fold, respectively) in the CL/Fr tissues, suggesting that all four genes may be involved in the CLP mutation in CL/Fr mice. Future gene expression studies through quantitative RT-PCR and regional expression analyses through immunohistochemistry will be performed to further test the expression of these clf1 and clf2 candidate genes. Supported in part by NIH/NCRR 5P20RR024206 (S.J.S) and R01-DK-064752 (SL).
Differential gene expression in mice with misexpression of six2 associated with frontonasal dysplasia

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We have previously described the Br mutant mouse displaying heritable frontonasal dysplasia. Linkage analysis mapped the mutation near the homeobox transcription factor six2, normally expressed in the facial mesenchyme during embryonic development. The purpose of this study is to determine expression patterns of six2, as well as possible upstream and downstream targets of six2, in the developing midface. The three sets of paired facial prominences (medial, lateral, and maxillary) of E11.5 embryos were dissected and RNA extracted for qPCR assays and Agilent microarray analysis. Medial nasal prominences (MNP) were also taken for cell culture. qPCR results indicated six2 expression is highest in the MNP and demonstrated haploinsufficient down-regulation in each of the three facial prominence sets in the Br mouse. Microarray results suggested the misregulation of several genes involved in a wide variety of genetic pathways. Further validation will be required to corroborate these microarray results, including qPCR, immunohistochemistry and RNA interference. Preliminary results using an in vitro knockdown of six2, performed on an MNP cell culture system utilizing siRNA, demonstrated a 65-70% knockdown of six2. These results may enable further in vitro work in order to elucidate a pathway in the developing midface involving six2. Supported by 1R01-DK-064752 (SL).
Genome-wide association study of three dimensional facial morphology identifies a variant in the PAX3 gene associated with nasion position

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We conducted a genome-wide association study of normal variation in 3D facial morphology in a population-based cohort. 3D high-resolution facial images were obtained in 4,747 15-year-old children from the Avon Longitudinal Study of Parents and Children, 22 landmarks identified and their x, y and z co-ordinates used to generate 54 parameters. These were tested for association in a discovery cohort comprising 2,185 individuals with genome-wide data available. We identified four associations in the discovery phase which met genome-wide significance (p<5x10^-8). We attempted to replicate these findings in a further 1,645 individuals from the same cohort. The association between rs7559271 (in an intron of PAX3) and the 3D distance between the nasion and the mid-endocanthion (n-men) was replicated (p=4x10^-7). In a combined analysis (p=4x10^-16) each G allele was associated with an increase in n-men of 0.39mm (explaining 1.3% of the variance). We deconstructed the 3D distance into one and two dimensional distances. Strong associations were observed in both the z and y dimensions (p=4x10^-9 and p=5x10^-8, respectively), with a much weaker association in the x dimension (p=0.006). The associations in the y and z dimensions were independent of each other, suggesting that the locus primarily influences growth and development in the yz plane. The PAX3 gene has previously been associated with Waardenburg syndrome, a condition involving characteristic facial morphologies including a flattened nasal bridge and widely spaced eyes. Our study suggests that less pathogenic (and more common) mutations within PAX3 may produce non-pathological variation in face shape within the normal range.
Genome-wide meta-analysis of nonsyndromic cleft lip with or without cleft palate (NSCL/P) identifies multiple new loci

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Nonsyndromic cleft lip with or without cleft palate (NSCL/P) is amongst the most common congenital birth defects. The etiology of this malformation, which involves environmental and genetic factors, has recently been enlightened by the discovery of six genetic susceptibility loci in genome-wide association studies (GWAS). To identify additional loci we conducted a meta-analysis of the two largest GWAS on NSCL/P, i.e. a case-control study of Central Europeans (Mangold et al, 2010) and a family-based study involving European and Asian trios (Beaty et al. 2010, data retrieved from dbGaP). Our analysis confirms all previously identified loci and identifies six new susceptibility regions for the European population (1p36, 2p21, 3p11.1, 8q21.3 13q31.1 and 15q22). Population-specific analysis revealed that five of them also play a role in the Asian population, suggesting that we have identified common genetic risk factors for NSCL/P. Candidate genes within these regions include SPRY2, THADA, PAX7 and EPHA3, opening new starting points for subsequent in-depth genetic and functional studies.
GROWTH OF THE SKULL AND BRAIN IN A MOUSE MODEL FOR APERT SYNDROME

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Craniofacial and neural tissues develop in concert throughout pre- and postnatal growth. Craniosynostosis syndromes, such as Apert syndrome (AS), are associated with specific phenotypes involving both the skull and the brain. Analysis of a mouse model for AS, the Fgfr2+/P253R Apert syndrome mouse, allows for the analysis of the effects of the P253R mutation on neural and skeletal tissues over the course of development. Previous work on this mouse model has demonstrated specific and localized effects on the developing brain and skull. The purpose of this study is to compare the Fgfr2+/P253R Apert syndrome mouse and unaffected littermates at two developmental time points, to determine whether growth patterns differ in brain and skull. 3D micro-magnetic resonance images and computed tomography scans were acquired from mice with the Fgfr2+/P253R mutation and their wild-type littermates at P0 (N=28) and at P2 (N=23). 3D coordinate data for 15 brain and 24 skull landmarks were collected using Amira© and Analyze 10.0© software and statistically compared using Euclidean Distance Matrix Analysis. Results demonstrate that the Fgfr2+/P253R mice show reduced growth in the cerebrum and the facial skeleton, while the height and width of the neurocranium and posterior regions of the brain show increased growth as compared to unaffected littermates. This localized correspondence of differential growth patterns in skull and brain point to their continued interaction through development, while also demonstrating that both tissues display divergent postnatal growth patterns as compared to their wild-type littermates.

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Phenotypic continuum in FGFR syndromic craniosynostosis? Evidence from human patients and mouse models

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Of the more than 180 craniosynostosis syndromes (CS) with a prevalence of 1/10,000 live births, many are caused by mutations in fibroblast growth factor receptors (FGFRs). The FGFR-related CS involve premature fusion of cranial sutures associated with craniofacial, neural, limb and visceral malformations. The precise definition and classification of FGFR-related CS can be difficult due to phenotypic variation within diagnostic categories, such that genetic testing is often required. However, there is no one-to-one correspondence between genetic mutations and phenotypes: a single mutation can result in different phenotypes, and mutations in different genes may produce similar phenotypes. We focus on a subset of CS caused by mutations in FGFR1, 2 and 3 (Apert, Crouzon, Pfeiffer and Muenke syndromes) to precisely quantify the phenotypic spectrum of these disorders based on geometric morphometric analysis of 3D landmark coordinates collected from CT reconstructions of the skull of human patients (N=37), unaffected individuals (N=20) and mouse models for CS (N=96 mutant; 109 non-mutant littermates). Our analyses suggest that there is correspondence between human and mouse data and that within both organisms the cranial morphologies associated with CS are distributed over a phenotypic continuum that ranges from no dysmorphology to various degrees of mild and severe dysmorphology. Individuals with Apert syndrome are the most severely affected, whereas individuals with Crouzon, Pfeiffer and Muenke syndromes display mild to severe dysmorphologies. Along this phenotypic spectrum fairly well defined diagnostic groups overlap due to high degrees of within-group variation, suggesting that common genetic and phenotypic variation underlie FGFR-related CS.
Proteins Regulation of Enamel Crystallographic Ultrastructure


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Enamel is a composite material that comprises an inorganic matrix composed of hierarchically organized carbonated-hydroxyapatite (HA) crystals, and an organic matrix mainly composed of the protein amelogenin. Understanding the relation between tooth enamel chemical components is of special interest for the interpretation of the variations in tooth development among humans. Accordingly, this study was designed to investigate how variations in the enamel proteins may affect its ultrastructure.

One-hundred extracted sound teeth were collected from adult patients attending McGill-Undergraduate Dental Clinic. FTIR and XRD were used to asses enamel chemical composition (protein content and degree of HA carbonization) and crystallography (crystal size, lattice parameters: a-axis and c-axis). The data obtained was analyzed for correlation, and statistical significance was set at P<0.05.

Tooth enamel protein content and crystallographic structure varied dramatically within the studied population. Enamel protein content was inversely correlated with its HA crystal size (R=-0.352, B=-19.4). Further analysis revealed that this correlation was not purely linear. Instead, it followed a curve; in which at specific enamel protein content, tooth enamel samples had the maximum HA crystal size. However, below or above that specific enamel protein content, teeth expressed smaller HA crystals [(R=0.32, B=271.4) and (R=-0.36, B=-15.1), respectively]. Moreover, the amount of tooth enamel protein was positively correlated with the carbonization degree of HA crystals (R=0.474, B=0.410).

From the present study, we conclude that tooth enamel proteins exhibited a dual behavioral effect on the size of HA crystals. Moreover, the degree of HA carbonization was also regulated by enamel proteins.
Fibroblast growth factors and their receptors (FGF-FGFRs) are critical in fundamental processes of craniofacial development, specifically with reference to bone development. Mutations in FGFRs have been identified as causative in numerous craniosynostosis syndromes. Crouzon syndrome is associated with nearly 50 known FGFR2 mutations, one of which is the FGFR2 C342Y mutation. Individuals with Crouzon syndrome show marked phenotypic variation but usually display premature closure of cranial suture(s), additional craniofacial malformations, as well as effects on other organ systems including respiratory disorders and auditory impairments. We used mice at postnatal day 0 (P0) of a mouse model for Crouzon syndrome, the Fgfr2C342Y/+ mouse, to analyze the impact of C342Y mutation on the negative spaces of the nasopharynx that develops within the intramembranous facial skeleton, and the inner ear structures that develop within the otic endochondral skeleton which is still cartilaginous at birth. Volumetric measurement using µMR indicated restriction of the nasopharynx of Fgfr2C342Y/+ mutant mice (n=8) compared to non-mutant littermates (n=11) but no difference in cochlear and semicircular canal volume. Global and regional shape variation in skull morphology were observed in mutant mice (n=28) relative to non-mutant littermates (n=31) using configurations of 3D landmark coordinates measured on µCT isosurfaces that quantify the facial skeleton, cranial base and cranial vault. Future work aims to determine whether differences in the effect of the FGFR2 C342Y mutation on these negative spaces are due to direct effects on the development of the structures, or effects on the types of bone in which they develop.
Reduced Bone Mass in Mice Lacking the Men1 Gene in Osteoblasts

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Homozygous inactivation of the multiple endocrine neoplasia type 1 (Men1) gene encoding menin in mice is embryonic lethal and fetuses exhibit clear defects in cranial and facial development. We have shown that menin has an important role in osteoblastogenesis and osteoblast differentiation by in vitro studies (JBC 2003). To further understand the physiological role of menin in bone development in vivo we are generating mouse models in which the expression of the Men1 gene is altered only in osteoblasts. Mice lacking Men1 exon 3 to 8 in osteoblasts driven by Osteocalcin-Cre (Men1 KO mice) displayed no differences in growth rate compared to WT littermates. In 9-month-old female mice, micro-CT revealed that trabecular bone volume and cortical bone thickness were significantly reduced in the Men1 KO mice. Histomorphometric analysis showed that bone volume/total volume, the numbers of osteoblast and osteoclast, as well as mineral apposition rate were significantly reduced in the Men1 KO mice. In mice overexpressing human menin cDNA in osteoblasts driven by a Col1a1 promoter (Men1 TG mice), at six months of age, the Men1 TG mice were not different from WT littermates in growth rate and bone mineral density by DXA. Taken together, depletion of menin in the osteoblast leads to decreased osteoblast and osteoclast numbers as well as impaired bone remodeling, resulting in a reduction in trabecular and cortical bone whereas overexpression has no effect at least in younger mice. Therefore, maintenance of menin expression and function in bone is important to avoid decreased bone mass.
Replication of GWAS Candidate Genes in Four Independent Populations Confirm the Role of Common Variants and Identifies the Contribution of Rare Variants in PAX7 and VAX1 in the Etiology of Non-syndromic CL(P)

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Background: Genome wide association studies (GWAS) of cleft lip with or without cleft palate (CL(P)) have identified several significant and near-significant genetic associations for non-syndromic CL(P) (Beaty et al. (2010). To replicate two of the near-significant GWAS signals, the present study investigated the role of both common and rare variants in the PAX7 and VAX1 genes.

Methods: Direct sequencing and TaqMan genotyping were carried out using GWAS markers in VAX1 and PAX7 on 5,421 individuals from Iowa, Japan, Philippines and Mongolia.

Results: TDT analysis showed strong associations with markers in VAX1 (rs7078160, p=1.46E-06 and rs475202 p=0.0008) in both Mongolian case and Japanese case-parent triads. Analysis using PLINK analyses suggested a possible maternal genotype effect for a VAX1 marker in Mongolian and Japanese combined CL(P) cases versus controls (rs7078160, p=9.7E-05, OR=2.33). A significant association with CL(P) was also observed in the Philippines case-parent triads for rs70781860 (p= 0.03). Furthermore, CL(P) males were mostly responsible for the effects in both Japanese and Mongolian populations (rs7078160, p=5.2E-05, OR=3.40). Conclusions: Our study replicated previous GWAS findings for markers in VAX1 across three independent Asian populations, and identified rare variants in PAX7 that may contribute to the etiology of CL(P). The role of these rare variants...
warrants further investigation through deep sequencing around these and other candidate genes.

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Meeting Location

McGill University, New Residence Hall, Ballroom A.,
3625 Avenue du Parc, Montréal, Québec

(location is marked by an A on the city map below)

Social Hour Location

Hielo Resto-Bar Terrasse
*(just adjacent to New Residence Hall)*
3575, Av Du Parc, Montréal, QC H2X 3P9
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Faculty of Dentistry, McGill University

The journal Genesis, John Wiley & Sons, Inc.

The journal Congenital Anomalies, Wiley-Blackwell