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Conference Report: The Society for Craniofacial Genetics and Developmental Biology 38th Annual Meeting

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The mission of the Society for Craniofacial Genetics and Developmental Biology (SCGDB) is to promote education, research, and communication about normal and abnormal development of the tissues and organs of the head. The SCGDB welcomes as members undergraduate students, graduate students, post doctoral researchers, clinicians, orthodontists, scientists and academicians who share an interest in craniofacial biology. Each year our members come together to share their novel findings, build upon, and challenge current knowledge of craniofacial biology.

Not even a hurricane could keep participants away from the 38th Annual Meeting of the Society of Craniofacial Genetics and Developmental Biology (SCGDB) held in Baltimore at the Johns Hopkins University and hosted, in part, by the Greenberg Center for Skeletal Dysplasias, the McKusick-Nathans Institute of Genetic Medicine and the Department of Pediatrics. Once again, the SCGDB meeting preceded the annual meeting of the American Society for Human Genetics and allowed for interactions among multiple investigators and trainees from around the world sharing a common interest in craniofacial biology. A new addition to this year's meeting was a dedicated clinical session, which, together with basic research sessions, provided insight into craniofacial genetics, development, disease and repair. Each session consisted of a plenary talk by prominent members of the field (Julie Hoover-Fong, Maximilian Muenke, Sally Moody, Warren Grayson and Dean Dessem),

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together with oral presentations chosen from submitted abstracts. In addition, a large poster session was held to further showcase the work being done in the craniofacial development and disease fields. Importantly, speakers included graduate students and postdoctoral fellows, highlighting a key objective of the Society—to promote its junior members and create an inclusive atmosphere that facilitates stimulating scientific discussions. In accordance with this, several trainee travel awards and poster prizes for both postdoctoral fellows and graduate students were made available through an R13 grant from the National Institute for Dental and Craniofacial Research. The meeting also received generous support from the scientific publications Developmental Dynamics and Genesis (the Journal of Genetics and Development), Fischer Scientific and VWR. The following trainees were recipients of poster prizes. In the post-doctoral scientist category, Kara Powder and Chris Percival tied for first place with Susan Motch Perrine in second. In the student category, first place went to Ryan Roberts, second to Karla Terrazas and third to Paul Pown Raj Iyannar. Congratulations to all for outstanding presentations.

Here, we present a brief overview of the 2015 meeting, followed by the abstracts of both oral and poster presentations. The 39th annual meeting of the SCGDB will be held August 3rd and 4th, 2016 in Boston immediately preceding the Society for Developmental Biology/ International Society for Developmental Biology joint meeting.

CLINICAL SESSION: CRANIOFACIAL DYSMORPHOLOGY: DIAGNOSIS, TREATMENT, REPAIR

The first speaker to kick off this new clinically oriented session was **Julie Hoover-Fong** (Johns Hopkins), who presented an overview of genetic medicine, particularly the role of medical genetics healthcare providers in multi-disciplinary cleft and craniofacial clinics. She discussed the structure of a comprehensive clinical genetics evaluation (e.g. 3 generation pedigree, teratogen exposures, medical history and physical exam) and the process of combining these data with addition studies (e.g. x-rays, echocardiogram) and tests (e.g. SNP array, single gene sequencing) to reach a unifying genetic or syndromic diagnosis for each patient. She and her colleagues completed a 3-year retrospective analysis of all patients in the Johns Hopkins Cleft Clinic. They compared the patients presenting diagnoses with that reached after the genetic medicine clinical encounter(s) and diagnostic workup, showing how healthcare and genetic counseling are dramatically improved with this comprehensive approach. **Edward Ahn** (Johns Hopkins) presented his approach to the diagnosis, quantitative and qualitative, and surgical decision-making for patients with all forms of craniosynostosis. He then shared several examples of successful cranial recontouring via endoscopic surgical methods and helmet therapy. Together, these procedures result in reduced scarring compared to traditional open craniectomy, while properly molding the cranial vault architecture. **Anand Kumar** (Johns Hopkins) then followed with a presentation on the surgical treatment of refractory obstructive sleep apnea caused by midface retrusion in individuals with Pierre Robin sequence and short stature skeletal dysplasias. He reviewed the overall process of distraction osteogenesis, which involves facial skeletal movements using a “top down” approach that reflects the normal spatial growth pattern of the craniofacial skeleton. **Richard Redett** (Johns Hopkins) concluded this session by presenting

a surgeon's perspective on cleft care in the 21st century, discussing the role of each specialty service in the multi-disciplinary care of these patients. Referring to facial difference in his patients, rather than deformity, he focused on how to achieve high patient satisfaction in both craniofacial form and function. Dr. Redett also emphasized the importance of engaging and educating parents of cleft lip/palate patients from the outset, as parental attitude directly impacts how the patient fares in the long run.

SESSION I: GENOMIC APPROACHES TO UNCOVER DISEASE MECHANISMS

This session focused on the power of genomics tools to reveal novel genes associated with craniofacial anomalies. The session began with a presentation by one of the plenary speakers, **Maximilian Muenke** (NHGRI), who reviewed the various craniosynostosis syndromes and their causes. This included Muenke syndrome, which is linked to a mutation in *FGFR3*, and is likely found in as many as 1/10,000 to 1/20,000 individuals. After examining 106 individuals with mutations in *FGFR3*, 84% were found to have craniosynostosis, while hearing loss, developmental delays, and intellectual disabilities were noted in 75%, 66% and 42% of the individuals, respectively. Dr. Muenke then presented data on one family in which *FGFR3* mutations caused significant differences in behavior, such as working memory, emotional control and inhibition, in affected individuals. Dr. Muenke also described work aimed at identify modifying factors in Muenke syndrome patients by comparing minimal versus severe pathologies.

The next speaker, **Marta Waberski** (NHGRI), outlined her findings from a case study in which a patient diagnosed with craniosynostosis was treated by an endoscopic craniectomy followed by cranial recontouring through helmet therapy. The patient, however, also presented with hearing loss and later some developmental delays. Whole exome sequencing revealed a frameshift mutation that caused a premature stop codon in *TCF12*. This gene encodes a class I basic helix-loop-helix (bHLH) protein that can form heterodimers with other bHLH proteins such as Twist1, mutations in which are known to cause craniosynostosis. It is intriguing to speculate that perhaps the phenotypes associated with this newly described craniofacial syndrome can be attributed to aberrant or even lost interactions of mutant TCF12 with other bHLH proteins. Importantly, TCF12 related craniosynostosis is now a recognized diagnosis and *TCF12* has now been integrated into clinical molecular testing for craniosynostosis. **Elizabeth Leslie** (University of Pittsburgh) then followed with a talk describing a large multi-ethnic (Europeans, Africans, Asians, Central/South Americans) genome-wide association study (GWAS) of cleft lip/palate that was conducted to identify additional risk factors. The GWAS identified *rhophilin2*, a gene encoding a RhoA binding protein that plays a role in actin dynamics, as a new factor for cleft lip/palate. Further analysis also revealed a novel association of *TANC2* with cleft lip/palate among individuals of European ancestry. These results suggest the importance of looking for population specific signals when searching for associations. To identify genetic modifiers, cleft lip and cleft lip/palate cases were compared, leading to the identification of the non-coding RNA LINC00922 as a significant risk factor for cleft lip. The last presentation of this session, given by **Deepti Anand** (University of Delaware), outlined a

new tool (systems tool for craniofacial expression-based gene discovery, or SysFACE) developed to prioritize candidate genes linked to craniofacial disorders and diseases identified in the group's own whole exome sequencing analysis of five individuals from a family of eight affected individuals. Through this analysis, Dr. Anand and others identified a novel variant of *ARHGAP29* in all five individuals in which a serine residue is replaced by a proline residue. Functional analysis of this variant in zebrafish revealed that it affects epiboly. Importantly, SysFACE will also allow researchers to prioritize candidate genes involved in craniofacial development and disease that have been identified in other databases such as Facebase.

SESSION II: COMPARATIVE MODELS OF CRANIOFACIAL DEVELOPMENT AND FUNCTION

This second session reinforced the use of basic research in different animals as a means to model human craniofacial disorders. The session commenced with a plenary talk by **Sally Moody** (George Washington University), who discussed her work to identify new candidates for branchio-oto-renal spectrum disorders (BOS/BOR) using *Xenopus laevis*. These disorders, while fairly rare, consist of mild craniofacial phenotypes but can have defects in the kidney and in the middle and inner ear, the latter leading to hearing loss. Because only 40% and 4% of patients possess mutations in *Eya1* and *Six1*, respectively, additional genes must play a role in the etiology of BOS/BOR. To provide insight into BOS/BOR, the Moody lab performed a microarray aimed at revealing downstream targets of Six1. One interesting candidate is identified in the microarray is *LOC1000037047*, which may encode a sulfotransferase. *LOC1000037047* is expressed broadly in the neural tube, neural crest, and preplacodal ectoderm, but expression later resolves to the olfactory placode, otocyst and branchial arches. Morpholino-mediated knock-down of *LOC1000037047* leads to the expansion and reduction of the neural and non-neural ectoderm, respectively, along with a loss of expression of many placode genes. Conversely, *LOC1000037047* overexpression caused the loss of the otocyst itself due to otic-specific genes not being expressed in the proper spatiotemporal pattern. Dr. Moody's group also sought to identify factors involved in modifying Six1 activity, and in doing so turned to the *Drosophila* embryo, where the original fly homolog of Six1, sine oculis, was described. Through a computational approach, 25 proteins were predicted to interact with sine oculis, and, perhaps not surprisingly, many of these were expressed in the frog ear and kidney. By applying additional criteria, the Moody lab then identified a protein called 2G4, which physically interacts with Six1 and leads to the loss of otocyst-specific genes upon its knock-down.

Jean-Pierre Saint-Jeannet (New York University) then presented his lab's work in establishing *Xenopus laevis* as an animal model for Nager syndrome, which is characterized by acrofacial dysostosis and is linked to mutations in *SF3B4* (splicing factor 3b, subunit 4), a component of the pre-mRNA spliceosomal complex. Because frogs and humans share over 80% identity of this protein, the Saint-Jeannet lab investigated *Sf3b4* spatiotemporal activity in the frog. *Sf3b4* is expressed in neural crest cells and later becomes enriched in the head and branchial arches. Interestingly, *Sf3b4* depletion through morpholinos leads to a loss of neural crest cell-specific gene expression (and thus neural crest cells). Consequently, neural

crest cell-derived cartilage is reduced upon loss of *Sf3b4*, phenocopying aspects of Nager syndrome. It is intriguing to contemplate how perturbation of a global process (splicing) can lead to such a specific phenotype (loss of neural crest cells). Continuing with the neural crest cell theme, **Elizabeth Schock** (Cincinnati Children's Hospital Medical Center, Bruggmann lab) presented data on how neural crest cells contribute to the oral-facial phenotypes associated with Oral-Facial-Digital (OFD) syndrome using the avian *talpid²* mutant. This animal model arises due to a mutation in a gene that encodes the centriolar protein calcium C2-dependent domain containing 3 (*C2CD3*). In the *talpid* mutant, neural crest cells are more dispersed due to decreased directional persistence, which reduces their ability to properly migrate and later differentiate. These results reveal a new role for a centriolar protein in the etiology of OFD. **Jacqueline Tabler** (University of Texas, Austin, Wallingford lab) next presented work on craniofacial development using the mouse as a model. In her examination of *Fuz* mutant mice, Dr. Tabler discovered that loss of *Fuz*, a CPLANE protein implicated in ciliopathies, leads to an increase in the number of neural crest cells and consequently the formation of a high-arched palate. This phenotype manifests from a loss of Gli repressor protein and subsequent expansion in *Fgf8* expression. Interestingly, *Fuz* mutants possess a single calvarial bone pair due to the bilateral presence of one mineralization center, indicating that the observed craniofacial phenotype is not due to craniosynostosis. Instead, Dr. Tabler's results reveal that loss of Gli3 repressor results in reduced parietal mesenchyme and a massive expansion of neural crest resulting in a large frontal bone. Cilia-mediated Gli signaling is essential for parietal morphogenesis. The last speaker of the session, **Hannah Edelman** (Johns Hopkins University, McCallion lab), presented data on the role of *Chromobox homolog 3* (*Cbx3*) in craniofacial development. This gene, which encodes for heterochromatin protein 1 gamma, was originally identified, along with four other candidates, to be deleted in a patient with facial dysgenesis. *Cbx3* protein is known to play important roles in the maintenance of transcriptional silencing, RNA processing and in DNA repair. To assess its function, the group turned to zebrafish, where it discovered two paralogs (*cbx3a*, *cbx3b*), both of which are expressed ubiquitously early in development, before becoming restricted to craniofacial structures. Using a CRISPR/Cas9-mediated knockout, the group discovered that although they could induce a frameshift mutation in *cbx3a*, which should lead to *cbx3a* nonsense-mediated decay, they observed no craniofacial phenotype. This was likely due to a compensatory increase in *cbx3b* expression, as has been observed in other instances in fish upon usage of CRISPR/Cas9. Additional studies in which both paralogs are depleted will provide more information about the role of *cbx3* genes in craniofacial development.

SESSION III: GENETIC INSIGHTS INTO CRANIOFACIAL DEVELOPMENT AND DISEASE

The third session focused on molecular mechanisms underlying the formation of craniofacial structures and how this translates to disease. **Dean Dessem** (University of Maryland) opened the afternoon session with a plenary talk on chronic overlapping pain conditions. In his presentation, Dr. Dessem revealed that 64% of patients with temporomandibular joint disorder (TMD) also suffer from irritable bowel syndrome (IBS), and that there is a high association between stress, TMD and IBS. Chronic overlapping pain conditions are more

prevalent in females versus males due to the likely involvement of estrogen level changes, not necessarily absolute value, throughout the menstrual cycle. Furthermore, Dr. Dessem's lab is pioneering the rat as an animal model for comorbid pain conditions such as TMD/IBS. To this end, Dr. Dessem's group uses ovariectomized rats, which are administered estradiol (to mimic estrogen fluctuations), followed by the introduction of an injury and stress. Visceral hypersensitivity and cutaneous mechanosensitivity reactions were then measured, and revealed an estrogen-dependent response for both. Furthermore, the onset of IBS and TMD is linked to the expression of *COMT*, a gene that encodes the enzyme catechol-O-methyltransferase. This enzyme has also been associated with an increased sensitivity to stress, further underscoring the crosstalk between stress and disease.

The second talk of this session, given by **Shachi Bhatt** (Stowers Institute for Medical Research, Trainor lab), discussed the role of *med23* in regulating mammalian craniofacial development. Originally identified through an ENU mutagenesis screen, *med23* encodes a protein subunit of the global Mediator transcription co-factor complex. A single point mutation in *med23* gives rise to embryos that die mid-gestation exhibiting deformation of the first arch and diminished neurogenesis. *Med23* mutants show normal specification and early patterning of the cranial placode and neural crest cell populations, but later, defects in the formation of the cranial nerves and craniofacial skeleton are observed. Specifically, placode cell-derived neurons exhibit an up-regulation of canonical Wnt signaling such that a partial rescue of cranial gangliogenesis occurs through titration of Wnt signaling back to wild-type levels. **Andrew Schiffmacher** (University of Maryland, Taneyhill lab) next presented data on a unique function for a cadherin proteolytic fragment during the cranial neural crest epithelial-to-mesenchymal transition (EMT). This EMT is essential to allow premigratory neural crest cells to become motile and later differentiate into appropriate cell types. Dr. Schiffmacher's data reveal that the proteolysis of a single cadherin in the chick embryo, Cadherin-6B, not only allows for an overall decrease in neural crest cell-cell adhesion, but also generates an intracellular C-terminal fragment (CTF2) that can bind to catenins and modulate transcription. Importantly, many of the targets of CTF2-mediated transcription include critical EMT effector genes, revealing an additional role for cadherin proteolysis during cranial neural crest cell EMT. **James Ferguson** (Case Western Reserve University School of Medicine, Atit lab) discussed how Wnt- β -catenin signaling in the cranial neural crest-derived supraorbital mesenchyme is important for both skull bone and dermis fate and to suppress the formation of cartilage. RNA-sequencing revealed that loss of β -catenin up-regulates many genes important for chondrogenesis (*Sox9*, *Col2a1*). Intriguingly, these genes are also targets of the Polycomb Repressive Complex 2 (PRC2), which includes the subunits EZH2, Suz12, and EED, in other systems. A "shower cap assay" of dissected cranial mesenchyme (in the presence or absence of β -catenin) showed no change in the transcript levels of *PRC2* components, indicating that the relationship between β -catenin and PRC2 might involve recruitment of PRC2 to specific genes. This hypothesis was validated through ChIP-sequencing of the cranial mesenchyme, which demonstrated the presence of PRC2 repressive marks chondrogenic genes such as the *Sox9* promoter. **Jennifer Fish** (University of Massachusetts, Lowell) concluded the session with a talk on the role of canalization during mouse facial development, and the influence of *Fgf8* on this process. Taking advantage of an allelic series of *Fgf8* mouse mutants, Dr. Fish presented

molecular, cellular and morphological data indicating a non-linear relationship between Fgf8 signaling and cell behavior and gene dosage. However, a linear relationship exists between *Fgf8* expression itself and *Fgf8* gene dosage. These results suggest the requirement for a threshold level of Fgf8 during normal craniofacial development, with only small deviations from this leading to morphological variations within individuals of similar genotypes. These data provide further evidence for the importance of tightly controlling Fgf8 signaling during development, which likely occurs through the deployment of several gene regulatory networks.

SESSION IV: MECHANISMS OF CRANIOFACIAL DISORDERS AND REPAIR

The final session of the meeting highlighted the various molecular mechanisms by which craniofacial disorders can arise, together with novel and innovative approaches by which to prevent and/or treat these disorders. The session began with a plenary talk by **Warren Grayson** (Johns Hopkins University), whose research focuses on designing craniofacial constructs for therapeutic uses. In his presentation, Dr. Grayson discussed the potential for using adipose-derived stem cells for bone regeneration in a process that involves direct implantation into patients in lieu of growing bone cells *ex vivo* followed by grafting. Isolated adipose-derived stem cells can form osteogenic, perivascular and endothelial cells and therefore provide the appropriate cell source. Although these cells can self-assemble vascular networks in the absence of exogenous growth factors, PDGF enhances their osteogenic differentiation. Taken together with a bioactive scaffold, adipose-derived stem cells were shown to increase bone formation in a mouse model with calvarial defects. Dr. Grayson's work also demonstrated the importance of timely oxygen delivery through the placement of oxygen "microtanks" to allow for vasculature assembly prior to transplantation.

In the second talk of the session, **Davide Komla-Ebri** (Paris Descartes University, working in the Legeai-Mallet lab, INSERM U1163, Institut Imagine) discussed how a pan-FGFR tyrosine kinase inhibitor, NVP-BGJ398, could be used to correct skull anomalies in a mouse model of achondroplasia that possesses a gain-of-function mutation in FGFR3. This inhibitor blocks the ATP binding site on the kinase domain of the receptor, preventing receptor phosphorylation and the subsequent activation of downstream signaling events. After only fifteen days of treatment with a low dose of the inhibitor (2mg/kg) initiated at P1, craniofacial skeletal defects were ameliorated and included a restoration of synchondroses and an increase in attributes such as foramen magnum size and nasal bone length. These findings will now be translated for use in children with achondroplasia. **Jeffrey Cesario** (New York University, Jeong lab) next presented data describing an anti-osteogenic role for LMX1B in mammalian calvaria development. Lineage tracing experiments in mouse revealed that LMX1B is observed in the dermis, meninges and sutures and therefore restricted from becoming bone. To tease out the mechanism behind this, a mesenchyme-specific knock-out of *LMX1B* was generated, resulting in mice born with severely dysmorphic heads and synostosis of multiple sutures, along with ectopic expression of osteogenic markers in the dorsal head mesenchyme. Conversely, mesenchyme-specific overexpression of *LMX1B* inhibited calvarial bone development because the supraorbital mesenchyme from which the calvaria arise failed to express osteogenic markers. Therefore,

LMX1B is an important player in controlling the balance between bone and soft tissue formation in the calvaria. **Cynthia Neben** (University of Southern California, Merrill lab) then described studies that shed new light on the etiology of the skeletal disorder Bent Bone Dysplasia Syndrome (BBDS). Osteoprogenitor cells normally reduce *ribosomal RNA* (*rRNA*) expression as they differentiate due to chromatin remodeling along the promoter. In BBDS, mutations in *FGFR2* enhance the nucleolar localization of its expressed protein, leading to activation of *rDNA* transcription through the maintenance of the euchromatic state, and a subsequent increase in ribosomes/polysomes and cell proliferation. Importantly, this can occur in pre-osteoblasts. Remarkably, this nucleolar-localized FGFR2 in BBDS can also inhibit the repression of *rDNA* transcription that is normally mediated by RUNX2, a master regulator of osteogenic differentiation, in the pre-osteoblast population. Collectively, these experiments define a molecular pathway by which an increase in pre-osteoblasts observed in BBDS patients can in turn lead to a decrease in the osteoblast population. The final talk of the meeting was given by **Rulang Jiang** (Cincinnati Children's Hospital Medical Center), who discussed his lab's findings on the identification of a Shh-Foxf-Fgf18-Shh molecular circuit during palatogenesis. Prior research indicated the importance of Shh signaling in regulating *Fox* gene expression during palate development, and that *Foxf2* null mice exhibit cleft palate. To gain a better understanding of the interrelationships between Shh and Fox, Dr. Jiang deleted *Foxf2* specifically in the neural crest-derived palatal mesenchyme and demonstrated a requirement for *Foxf2* during palate formation. Intriguingly, this *Foxf2* mutant exhibits increased *Fgf18* expression, as well as a reduction in *Shh* expression, in anterior and posterior subdomains of the palatal shelves. Interestingly, *Foxf1* and *Foxf2* share some overlapping expression domains, and inactivation of both genes in the neural crest caused ectopic *Fgf18* expression, and a striking loss of *Shh* throughout the entire palatal epithelium. These results suggest a regulatory feedback loop in which proper palate formation relies upon the activity of Shh, Foxf, and Fgf18.

ORAL PRESENTATION ABSTRACTS

PODIUM PRESENTATIONS IN ORDER OF APPEARANCE (SPEAKER IS UNDERLINED)

These talks are summarized in the proceeding section.

Clinical Session: Craniofacial Dysmorphologies: Diagnosis, Treatment, Repair

—Julie Hoover-Fong

All Patients with Orofacial Clefing Deserve a Medical Genetics Evaluation

Ed Ahn

Endoscopic Surgery for Craniosynostosis

Anand Kumar

Advances in Pediatric Sleep Apnea: Treatment of Facial Skeletal Hypoplasia using Distraction Osteogenesis

Richard Redett

Multi-disciplinary Cleft Clinic Care

Session 1 : Genomic Approaches to Uncover Diseases Mechanisms—Plenary
Speaker Maximilian Muenke

NHGRI, National Institutes of Health, Bethesda, Maryland

*Dr. Muenke's talk is summarized in the proceeding section.

Marta Waberski

Expanding the phenotype of patients with TCF12 mutations, bilateral coronal craniosynostosis and external auditory canal atresia: Marta B. Waberski¹, Natalie Beck², Amir Dorafshar², Edward Ahn², Julie Hoover-Fong²

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Craniosynostosis is the premature fusion of cranial sutures that affects ~1 in 2500 children. Normal brain growth in the context of deficient cranial growth can deform the skull and may increase intracranial pressure, predisposing to neurodevelopmental problems. Craniosynostosis is a heterogeneous disorder with multifactorial etiologies including single gene defects and environmental factors. Coronal sutures are the second most common sutures affected in craniosynostosis. Recently, heterozygous mutations in *TCF12* have been associated with predominant coronal craniosynostosis plus facial asymmetry, flat facies, hearing loss, external ear malformations, hand/foot anomalies and decreased penetrance. Our case further supports a role of *TCF12* in cranial bone remodeling illustrated by a patient with bilateral coronal craniosynostosis and external auditory canal atresia. Craniosynostosis was suspected in our patient shortly after birth. Clinical examination revealed brachycephaly, facial asymmetry with an abnormal contour of the forehead, midfacial hypoplasia and right external auditory canal atresia. A CT scan confirmed bilateral coronal suture fusion and right external auditory canal atresia, but also middle ear cavity opacification and asymmetric volume loss of the left temporal and frontal lobes. She underwent an endoscopic craniectomy at one month of age followed by cranial recontouring via helmeting. ABR testing at three months of age revealed severe hearing loss on the right with normal hearing on the left. A previously reported heterozygous mutation in *TCF12* (c. 1491dupT; p.V498Cfs*12) was found by whole exome sequencing in our patient which was inherited from her unaffected father. Our patient illustrates the expanding phenotypic spectrum in a newly described craniofacial syndrome, caused by mutations in *TCF12*.

Elizabeth Leslie

Multi-ethnic genome-wide association studies of CL/P identify novel risk genes and evidence for genetic modifiers: Elizabeth J. Leslie¹, John R. Shaffer¹, Jenna C. Carlson¹, Eleanor Feingold¹, Cecelia Laurie², Kim Doheny³, Katherine Neiswanger¹, Lina Moreno⁴, Andrew Lidral⁴, Andrew Czeizel⁵, Carmencita Padilla⁶, Frederic Deleyannis⁷, L. Leigh Field⁸, George Wehby⁴, Alexandre Vieira¹, Eduardo Castilla⁹, Azeez Butali⁴, Jacqueline

Hecht¹⁰, Carmen J. Buxo¹¹, Kaare Christensen¹², Lian Ma¹³, Jeffrey C. Murray⁴, Seth M. Weinberg¹, Mary L. Marazita¹

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CL/P, i.e. nonsyndromic cleft lip (CL) with or without cleft palate (CP), is one of the most common birth defects in humans, affecting approximately 1 in 700 newborns. CL/P is phenotypically heterogeneous and has a complex etiology caused by genetic and environmental factors. Historically, CL and CL plus CP (CLP) have been considered to have a common genetic etiology and are typically combined for genetic analyses. Previous genome-wide association studies (GWAS) have identified at least 15 risk loci for CL/P. We conducted a GWAS using 823 cases, 1700 controls, and 1319 complete case-parent trios with European, Asian, or African ancestry, and admixed populations from Central and South America. Our GWAS revealed a novel association on 19q13 near *RHPN2*, a gene involved in organizing the actin cytoskeleton ($p=4.17 \times 10^{-8}$). Other regions reaching genome-wide significance were 1p22 (*ARHGAP29*), 1q32 (*IRF6*), 8q24, and 17p13 (*NTN1*), all reported in previous GWAS. In addition, we found evidence of association in other previously reported CL/P GWAS regions although not at a genome-wide level of significance. Stratification by ancestry group revealed a novel association with a region on 17q23 near *TANC2*, a gene required for early embryonic development in mouse, ($p=1.41 \times 10^{-8}$) among individuals with European ancestry. Finally, we performed a case-only analysis contrasting CL cases ($n=450$) with CLP cases ($n=1692$) to search for genetic modifiers. This identified a region on 16q21 containing non-coding RNA LINC00922 as a modifier significantly increasing risk for CL ($p=2.37 \times 10^{-8}$). In summary, we have identified novel CL/P risk factors and suggest new genes involved in craniofacial development.

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Deepti Anand

Exome sequencing in a Multiplex Family with Cleft Palate Only Identifies a Novel Damaging Mutation in ARHGAP29: Azeez Butali¹, Tamara Busch², Lord Gowans¹, Huan Liu³, Deepti Anand⁴, Salil A. Lachke⁴, Nichole Nidey², Brad Amendt⁵, Robert Cornell³, Jeffrey Murray²

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Background: With advances in genomics research and availability of next generation sequencing approaches, scientists are now able to identify risk loci across the exome and the genome. In the current study, we present findings from an exome study conducted in a multiplex family with cleft palate only (CPO). **Methods:** Exome sequencing was conducted in 5 individuals with high quality DNA in a family of 8 affected individuals. The Illumina HiSeq 2000 was used for sequencing and Agilent Sure Select for enrichment capture. Quality control (QC) steps included aligning raw sequence to the human genome build hg19 using BWA software, SNP and In/Dels analyses using PICARD, SAMTOOLS and GATK. WANNVAR was used to functionally annotate the SNPs and In/Dels. Several filtration methods were applied in order to identify variants with minor allele frequencies 1% in 1000genomes, EVS and dbSNP138 including clinically associated variants that segregate in affected individuals. The bioinformatics tool *SysFACE* (*S*ystems tool for craniofacial expression-based gene discovery) was used to prioritize cleft candidates from our list of variants. Sanger sequencing was used to validate identified variants in affected and unaffected relatives. **Results:** A yield of 3.4Gbases per sample passed the quality control step. A missense variant predicted to be in exon 15 of *ARHGAP29* (pSer552Pro) was identified. This variant segregates in all 5 individuals. The pSer552Pro variant is novel and not in all of the known databases and has never been previously reported. This variant was validated using Sanger sequencing and none of the 4 unaffected individuals (a sibling and 3 unrelated individuals) including a CEPH control carried the variant. However, one of the unaffected siblings carried the variant. A functional assay in zebrafish embryos showed that the pSer552Pro variant lacked activity possessed by the reference variant. We also identified 2 clinically associated variants in *FRGB3* gene (rs145018822 and rs201563622) previously reported for malignant melanoma in the ClinVar study. This study illustrates the power of exome sequencing to reveal rare variants that are pathogenic for CPO.

Session 2: Comparative Models of Craniofacial Development and Function— Plenary Speaker Sally Moody

George Washington University, Washington, D.C.

*Dr. Moody's talk is summarized in the proceeding section.

Jean-Pierre Saint-Jeannet

Sf3b4-depleted Xenopus embryos, a model to study the pathogenesis of craniofacial defects in Nager syndrome: Arun Devotta¹, Hugo Juraver-Geslin¹, Jose Antonio Gonzalez^{1,2}, Chang-Soo Hong³ and Jean-Pierre Saint-Jeannet¹

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Mandibulofacial dysostosis (MFD) is a human developmental disorder characterized by defects of the facial bones, particularly the maxilla, mandible, and zygomatic complex, associated with malformation of the middle ear ossicles. Acrofacial dysostosis combines

many features of MFD with a variety of limb anomalies. The most common form of acrofacial dysostosis is Nager syndrome. Mutations in *SF3B4* (splicing factor 3b, subunit 4) gene, which encodes a component of the pre-mRNA spliceosomal complex, were recently identified as a cause for Nager syndrome, accounting for 60% of affected individuals. Nothing is known about the cellular pathogenesis underlying Nager type MFD. Here we describe the first animal model for Nager syndrome, generated by knocking down Sf3b4 function in *Xenopus laevis* embryos, using morpholino antisense oligonucleotides. Our results indicate that Sf3b4-depleted embryos show reduced expression of the neural crest specific genes *Snail2* and *Sox10*, associated with a broadening of the neural plate. At the tailbud stage, these embryos had a decreased number of neural crest cells in the pharyngeal arches. Later in development, Sf3b4-depleted tadpoles exhibited hypoplasia of neural crest-derived craniofacial cartilages, phenocopying aspects of the craniofacial skeletal defects seen in Nager syndrome patients. With this animal model we are poised to gain important insights into the etiology and pathogenesis of Nager type MFD, and to identify the molecular targets of Sf3b4. This work is supported by NIH/NIDCR R01DE014212.

Elizabeth Schock

Using the avian mutant talpid² as a disease model for understanding the oral-facial phenotypes of Oral-facial-digital syndrome: Elizabeth N. Schock^{1,2}, Ching-Fang Chang^{1,2}, Jaime N. Struve^{1,2}, Ya-Ting Chang^{1,2}, Julie Chang³, Mary E. Delany⁴ and Samantha A. Brugmann^{1,2}

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Oral-facial-digital syndrome (OFD) is a ciliopathy characterized by oral-facial abnormalities including cleft lip/palate, broad nasal root, dental anomalies, micrognathia and glossal defects. In addition, these patients have several other characteristic abnormalities typical of a ciliopathy including polysyndactyly, polycystic kidneys and hypoplasia of the cerebellum. Recently, a subset of human OFD cases have been linked to mutations in the centriolar protein, calcium C2-dependent domain containing 3 (C2CD3). Our previous work identified mutations in *C2CD3* as the causal genetic lesion for the avian *talpid²* mutant. Based on this common genetic etiology, we re-examined the *talpid²* mutant for phenotypes characteristic of OFD. We found that all common phenotypes were conserved between human OFD patients and avian *talpid²* mutants. In light of this finding, we utilized the *talpid²* to examine the cellular basis for the oral-facial phenotypes present in OFD. Specifically, we examined the development and differentiation of cranial neural crest cells (CNCCs) when C2CD3-dependent ciliogenesis is impaired. Our studies suggest that, whereas disruptions of C2CD3-dependent ciliogenesis do not affect CNCC specification or proliferation, CNCC migration and differentiation are disrupted. Loss of C2CD3-dependent ciliogenesis affects the dispersion and directional persistence of migratory CNCCs. Furthermore, loss of C2CD3-dependent ciliogenesis results in dysmorphic and enlarged CNCC-derived facial cartilages.

These findings suggest that aberrant CNCC migration and differentiation could contribute to the pathology of oral-facial defects in OFD. This work is supported by NIH grant R00-DE01985 (S.A.B) and C.T.O funds from the Cincinnati Children's Research Foundation (S.A.B).

Jacqueline Tabler

Excess neural crest drives absent or reduced parietal bones after cilia or Gli3

perturbation: Jacqueline M. Tabler¹, Christopher P. Rice¹, Karen J. Liu^{2*}, and John Wallingford^{1, 3}

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The skull is essential for protecting the brain from damage and in mammals two bone pairs, frontal and parietal, encase the forebrain. However, the morphogenetic events that drive frontal or parietal bone development remain poorly understood. Using mouse models, we show here that defective cilia-dependent Gli signaling elicits an expansion of neural crest-derived parietal bone mesenchyme that can disrupt morphogenesis of the mesoderm-derived parietal bones. Surprisingly, we find that parietal bones are essentially absent in ciliopathic mutant mice. This loss of parietal bones is interesting because in cursory analyses, the phenotype appears similar to coronal synostosis. However, embryological and genetic fate-mapping experiments demonstrate that the defect arises through a distinct and novel mechanism. These findings identify defective parietal morphogenesis as a novel component of the spectrum of congenital cranial anomalies. To better understand such cranial defects, we have developed a novel live imaging platform with which we can directly image the behavior of living osteoblasts in intact skulls using time-lapse 3D microscopy. We will present evidence that normal skull morphogenesis involves a combination of oriented cell divisions, progressive differentiation and individual cell migration. This imaging technique provides a novel platform for understanding the cell biology of cranial morphogenesis, as well as a system for testing potential therapies of defective skull growth. J.M.T. is supported by a National Research Service Award from the NIDCR F32DE023272. This work was supported by funding from the BBSRC (BB/I021922/1) to K.J.L., a Wellcome Trust Value in People Award to K.J.L. and J.M.T., and by grants from the NIGMS and the NHLBI to J.B.W. J.B.W. is an Early Career Scientist of the HHMI.

Hannah Edelman

Cbx3 and its role in craniofacial development: zebrafish as a model system for testing dysmorphology candidate genes: Hannah E. Edelman¹, Courtney E. Woods¹, Julie E. HooverFong², Andrew S. McCallion¹

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With the wide range of craniofacial abnormalities seen in children, it is important to have an efficient pipeline to identify and test the candidate genes involved in these developmental anomalies. In 2003, Hoover Fong et al. reported a patient with severe facial dysgenesis and a deletion of 7p15.1p21.1. Using a SNP array from a different patient with an overlapping deletion but nonoverlapping phenotype, we found the region uniquely deleted in our patient with facial dysgenesis. We are systematically determining the roles of the five candidate genes in this narrowed region beginning with *cbx3* (chromobox homolog 3), whose protein product (heterochromatin protein 1 gamma, HP1 gamma) is thought to be involved in the switch from euchromatin to heterochromatin. In zebrafish, *cbx3* has been duplicated during evolution yielding *cbx3a* and *cbx3b* paralogs. Wholemount *in situ* hybridization of zebrafish embryos confirmed a probable role for *cbx3a* and *cbx3b* in the development of craniofacial structures, so we used CRISPR/Cas9 technology to create a line of fish with a two base pair deletion in the first exon of *cbx3a*. This frameshift mutation results in a premature termination codon and nonsense-mediated decay. We are currently phenotyping the offspring of this line using acid free double staining to examine the developing craniofacial cartilage and bone. Although our data are preliminary, they are suggestive of a requirement for *cbx3a* in normal facial development. We are using this work as a platform from which to build a systematic pipeline for the analysis of the genetic architecture of craniofacial anomalies. Funding: The Alan and Kathryn Greenberg Center for Skeletal Dysplasias; Fondation LeDucq.

Session 3: Genetic Insights into Craniofacial Development and Disease— Plenary Speaker Dean Dessem

University of Maryland, College Park, Maryland

*Dr. Dessem's talk is summarized in the preceding section.

Shachi Bhatt

Ubiquitously expressed Mediator subunit, Med23, is required for development of mammalian craniofacial structures and regulation of WNT signaling during cranial neuron differentiation: Shachi Bhatt¹, Lisa Sandell², Karla Terrazas¹, Ahn Youngwook³, Robb Krumlauf¹, Paul Trainor¹

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The vertebrate head is an assemblage of tissues that originate from all three germ layers. Of special importance are ectodermally-derived cranial placodal and neural crest cells, which form majority of the cranial nerves and craniofacial bone and cartilage. In an ENU screen aimed at identifying novel regulators of mammalian craniofacial development, we

discovered a point mutant in a gene called *med23*, which displays defects in cranial nerve and craniofacial development. *Med23* is a ubiquitously expressed gene, which encodes a protein subunit of the global transcription co-factor complex, Mediator. Detailed analyses in *med23-point mutant* embryos suggest that early specification and patterning of cranial placodes and neural crest is unaffected, however, loss of *med23* results in near-complete absence of placode-derived cranial neurons and severe craniofacial skeletal defects, including cleft palate and aberrant Meckel's cartilage development. Interestingly, the role of *med23* in neuronal differentiation of cranial placodes is associated with elevated levels of canonical WNT signaling, genetic suppression of which partially restores cranial nerve formation. Studies are underway to determine if Med23 has a similar role in regulating WNT signaling during craniofacial skeletal development. This work displays a surprisingly tissue-specific role for the ubiquitously expressed transcription co-factor, Med23 during cranial placodal and cranial neural crest cell differentiation, and highlights a previously unexplored link between general transcription machinery, Mediator, and regulation of conserved signaling pathway, canonical WNT signaling. This work is supported by the Stowers Institute for Medical Research.

Andrew Schiffmacher

Cadherin-6B soluble C-terminal fragments regulate EMT effector genes in chick cranial neural crest cells: [Andrew Schiffmacher](#)¹, Lisa A. Taneyhill¹

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At the onset of the epithelial-to-mesenchymal transition (EMT), many cells proteolytically process cadherins to disassemble cell-cell junctions and generate cleavage products with unique functions. Chick premigratory cranial neural crest cells (NCCs) downregulate existing Cadherin-6B (Cad6B) levels through multiple mechanisms, including proteolysis, before completing EMT, migrating throughout the head, and differentiating into cell types that form craniofacial tissue, sensory ganglia, and odontoblasts. Cad6B is serially processed by ADAM metalloproteinases and γ -secretase to generate shed N-terminal ectodomains and soluble intracellular C-terminal fragments (CTF2). Interestingly, Cad6B CTF2 overexpression in premigratory cranial NCCs prematurely reduces Cad6B levels, suggesting that CTF2 may participate in a regulatory loop to promote disassembly of Cad6B-containing adherens junctions and subsequent EMT. Our findings indicate that β -catenin is the primary interactor of CTF2, with elevated levels of CTF2 in NCCs causing a redistribution of β -catenin from the membrane to the cytosol. Migratory NCCs generated from premigratory NCC explants overexpressing CTF2 also display CTF2-dependent nuclear localization of β -catenin. In addition, co-immunoprecipitation experiments confirm a CTF2/ β -catenin direct interaction in both the cytosol and nucleus. Finally, transient overexpression of Cad6B CTF2 in NCCs significantly upregulates *β -catenin*, *CyclinD1*, and *Snail2* expression, and this is dependent on a CTF2/ β -catenin interaction since a CTF2 β -catenin-binding mutant fails to alter gene expression. Future experiments involving this mutant will clarify if these results are due to CTF2-mediated modulation of Wnt/ β -catenin signaling. Collectively, our data reveal that Cad6B proteolysis promotes expression of EMT effector genes, providing insight into the role of CTF2 molecules in EMTs underscoring development and disease. This work

is supported by grants to AS (NIH F32DE022990) and LAT (NSF IOS-0948525 and ACS CSM-127240).

James Ferguson

Wnt/ β -catenin activity-dependent Polycomb repression in craniofacial development:

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Proper craniofacial development involves complex spatial and temporal genetic interactions in the cranial neural crest cells (CNC) to allow for differentiation into various lineages. Within the CNC derived supraorbital cranial mesenchyme, Wnt/ β -catenin signaling is required for proper skull bone and dermal fate selection. In the absence of β -catenin, multiple chondrogenic genes are up-regulated followed by ectopic cartilage formation. How β -catenin activity suppresses the cartilage fate is unclear, however. Recently, we discovered that multiple genes up-regulated in the absence of β -catenin, such as *Sox9* and *Col2a1*, are also targets of the Polycomb Repressive Complex (PRC2) leading to the hypothesis that β -catenin requires PRC2 to regulate cell fate selection. In the mesenchyme, the components of PRC2 are present and active. However, the global expression level of these components does not change following conditional deletion of β -catenin indicating PRC2 and β -catenin are required on a gene specific level. Following chemical inhibition of EZH2 function in vitro, both *Sox9* and *Col2a1* are up-regulated further supporting gene specificity. Additionally, the PRC2 repressive mark, H3K27me3, is enriched on the *Sox9* locus in the cranial mesenchyme by ChIP-sequencing. Current studies are investigating to whether Wnt/ β -catenin activity is required for recruitment of PRC2 to its target genes. These findings provide new mechanistic insight into the mechanism by which β -catenin regulates lineage restriction and provides new insights into how epigenetic silencing and Wnt/ β -catenin signaling interact to promote proper lineage identity of the supraorbital cranial mesenchyme. This work was supported by the University Hospitals Musculoskeletal training grant NIH T32 AR007505 to JF, and R01-DE01870 to RPA.

Jennifer Fish

FGF8 signaling and canalization during mouse facial development: Jennifer L. Fish¹, Rebecca M. Green², Francis Smith², Benedikt Hallgrímsson², and Ralph S. Marcucio³

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Recent models of craniofacial development suggest that disease phenotypes are part of a continuous range of morphological variation, but mechanisms generating this variation are largely unknown. We quantified molecular, cellular, and morphological data from mouse embryos with 5 genotypes altering *Fgf8* dosages: *Fgf8*^{+/+}, *Fgf8*^{Neo/+}, *Fgf8*^{Delta/+}, *Fgf8*^{Neo/Neo}, *Fgf8*^{Delta/Neo} at E10.5 and P0. *Fgf8* is a signaling factor expressed in epithelial domains surrounding the mesenchyme of the developing face. Mice heterozygous for *Fgf8*

are phenotypically normal, however, they exhibit more variance in facial shape than WT individuals. Reduction in *Fgf8* dosage below 40% of WT levels causes micrognathia and midfacial anomalies and, similar to human disease, results in high levels of variation. Similarly, we find a non-linear relationship between Fgf8 signaling (as reflected in *Pea3* and *ERM* gene expression) and cell behavior (as reflected by percentage of apoptotic cells) and gene dosage at E10.5. However, we have found that *Fgf8* expression decreases linearly with *Fgf8* dosage. For example, *Fgf8*^{Delta/+} heads express roughly half the amount of *Fgf8* as wild-type heads. These data suggest that a threshold level of *Fgf8* expression is required to maintain signaling and promote normal morphogenesis. Thus, when expression levels approach the threshold, small fluctuations in protein concentrations may result in significant morphological variation even in individuals of similar genotypes. Taken together with our previous work with the *Tfap2a* mutant mouse, in which abnormally high Fgf8 signaling also causes mid-facial defects, our research suggests that Fgf8 signaling is tightly regulated during development, and likely involves multiple gene regulatory networks. This work was supported by NIH R01-DE019638 and R01-DE021708 to R.M. and B.H.

Session 4: Mechanisms of Craniofacial Disorders and Repair—Plenary Speaker Warren Grayson

Johns Hopkins University, Baltimore, Maryland

*Dr. Grayson's talk is summarized in the preceeding section.

Davide Komla-Ebri

Correction of skull anomalies in an achondroplasia mouse model with the tyrosine kinase inhibitor NVP-BGJ398: Davide Komla-Ebri¹, Emilie Dambroise¹, Ina Kramer², Catherine Benoist-Lasselin¹, Nabil Kaci¹, Ludovic Martin¹, Diana Graus Porta², Michaela Kneissel², Arnold Munnich¹, Federico Di Rocco¹, Martin Biosse Duplan¹, Laurence Legeai-Mallet¹

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Missense mutation localized in the *FGFR3* (*Fibroblast Growth Factor Receptor 3*) gene is responsible for the most frequent form of dwarfism: achondroplasia (ACH). *FGFR3* mutation induces an increased phosphorylation of this tyrosine kinase receptor causing over-activation of its downstream signaling pathways leading to defective endochondral and membranous ossification. ACH patients exhibit skull anomalies such as a larger cranium with prominent forehead, hypoplasia of the midface, non-ossified gaps in frontal bones, loss of synchondroses and reduced foramen magnum. Since ACH is due to FGFR3 over-activation, the use of selective small molecule Tyrosine Kinase Inhibitors (TKIs) to arrest FGFR3 phosphorylation seems to be a relevant therapeutic approach. For this reason we performed experiments with the pan-FGFR TKI "NVP-BGJ398". *In vitro*, NVP-BGJ398 reduced FGFR3 phosphorylation and inhibited FGFR3 downstream signaling pathways, MAPKs and PLCγ, in transfected chondrocytes and ACH chondrocyte lines. Studying calvaria (18.5 dpc) of fetal mutant mice mimicking ACH (*Fgfr3*^{Y367C/+}) we observed that NVP-BGJ398 at 100 nM corrected the calvarial non-ossified gap defect. Moreover, we

demonstrated that a low dose of NVP-BGJ398 (2 mg/kg), was able to improve the craniofacial skeleton after only 15 days of treatment. μ CT analyses demonstrated that treatment with NVP-BGJ398 prevented synchondroses loss, increased the foramen magnum size and modified skull anomalies (anteroposterior length, width, nasal bone length). In conclusion, the cranial hallmarks of ACH were largely improved with NVP-BGJ398. Our results have successfully demonstrated a rationale for targeting FGFR3 with TKI for the treatment of children with ACH. This program received a state subsidy managed by the National Research Agency under the “Investments for the Future” program bearing the reference ANR-10-IHU-01 and the “ANR-ATAK 2013” program.

Jeffrey Cesario

Role of LMX1B transcription factor in mammalian calvaria development: Jeffrey Cesario¹, Andre Landin Malt¹, Michael Khairallah¹, Asma Almaidhan^{1,2}, Veronica Choi¹, Jong Uk Chung¹, Mina Kim¹, Randy Johnson³ and Juhee Jeong¹

¹Department of Basic Science and Craniofacial Biology, New York University College of Dentistry, New York, NY, ²Department of Orthodontics, New York University College of Dentistry, New York, NY, ³Department of Biochemistry and Molecular Biology, University of Texas MD Anderson Cancer Center, Houston, TX

Normal development of the calvaria requires generating and maintaining a fine balance between its osseous components (calvarial bone) and non-osseous components (sutures and fontanels). Abnormalities in the calvaria due to the perturbation of this balance are a major class of birth defects in humans, including craniosynostosis. *Lmx1b* encodes a LIM-domain homeodomain transcription factor, and its mutation leads to Nail Patella syndrome in humans. While *Lmx1b*^{-/-} mutant mice had severe calvaria defects, it had not been clear whether they were secondary to the brain deformity or due to the direct role of LMX1B in head mesenchyme precursors of the calvaria. To distinguish the two possibilities, we deleted *Lmx1b* in the head mesenchyme using *Prrx1-Cre*. *Prrx1-Cre;Lmx1b*^{fllox/-} mutants were born with a severely dysmorphic head and synostosis of multiple sutures. During normal development of the calvaria, bone forms from the head mesenchyme just above the eye (supra-orbital mesenchyme), whereas only soft tissues such as the suture and dermis arise from the dorsal head mesenchyme. However, *Prrx1-Cre;Lmx1b*^{fllox/-} mutants showed ectopic osteogenesis in the dorsal head mesenchyme. In the converse experiment, overexpression of LMX1B in the head mesenchyme inhibited calvarial bone development. The supra-orbital mesenchyme of these mutants failed to express osteogenic markers, and they also did not form the mesenchymal condensations for the bone. Based on these results, we conclude that LMX1B suppresses osteogenesis in the head mesenchyme to regulate the balance of the bone and soft tissue in the calvaria. This work was supported by R03DE023617.

Cynthia Neben

Nuclear FGFR2 regulates the epigenetic state of rRNA chromatin during osteoblast differentiation: Cynthia L. Neben¹, Xiaojing Mao^{1,2}, and Amy E. Merrill^{1,2}

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Regulation of ribosome biogenesis is essential for cell proliferation and is tightly linked with cell commitment. Our studies in osteoprogenitor cells show that ribosomal RNA (rRNA) expression is down-regulated during osteoblast differentiation, and preliminary data suggests this is a result of chromatin remodeling at the rDNA promoter. Our previous analysis of the skeletal disorder Bent Bone Dysplasia Syndrome (BBDS) suggests that Fibroblast Growth Factor Receptor 2 (FGFR2) is a key regulator of rDNA transcription in the nucleolus during bone development. The unique BBDS mutations in the transmembrane domain of *FGFR2* reduce receptor levels at the plasma membrane, decreasing canonical FGF signaling, and enhance nucleolar localization the receptor. This augments a normal function for FGFR2 in the nucleolus where it directly activates rDNA transcription by interacting at the rDNA promoter with FGF2 and UBF1. Preliminary data suggests that nucleolar FGFR2 promotes rDNA transcription by maintaining the euchromatic state of rDNA, resulting in an increased production of ribosomes. Enhanced levels of ribosome biogenesis are directly linked to increased cell growth and proliferation. Correspondingly, we found that the BBDS mutations also increase proliferation in preosteoblasts. Our studies also show that nucleolar FGFR2 blocks the transcriptional repressor activities of RUNX2, the master regulator of osteoblast differentiation, at rDNA. Likewise, in BBDS, we found that RUNX2 nucleolar occupancy is blocked in preosteoblasts. Together this data suggests a mechanism through which altered nucleolar FGFR2 activity in BBDS may increase the osteoprogenitor cell population while decreasing the number of osteoblasts in affected individuals. This work is supported by NIH#1R01DE025222; the March of Dimes Basil O'Connor Starter Scholar Award #5-FY12-166 and Gene Discovery and Translational Research Grant #6-FY15-233; NIH 5T32HD060549-02.

Rulang Jiang

A Shh-Foxf-Fgf18-Shh molecular circuit regulating palate development: Rulang Jiang

Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA

Cleft palate is among the most common birth defects in humans. Whereas human genetic studies and analyses of mutant mouse models have identified many genes associated with cleft palate phenotypes, the molecular mechanisms regulating palatogenesis remain poorly understood. Previous studies have shown that Shh signaling plays critical roles in palate development and regulates expression of several members of the Forkhead-box (Fox) family transcription factors, including Foxf1 and Foxf2, during craniofacial and palate development. Although targeted disruption of *Foxf2* has been shown to cause cleft palate in mice, whether Foxf2 plays an intrinsic role in and how Foxf2 regulates palate development remain to be elucidated. Using Cre/loxP-mediated tissue-specific gene inactivation in mice, we show that Foxf2 is required in the neural crest-derived palatal mesenchyme for normal palatogenesis. We show that expression of *Foxf2* partly overlaps with that of *Foxf1* in the developing palatal mesenchyme and that the *Foxf2*^{-/-} mutant embryos exhibit ectopic

activation of expression of *Fgf18* and concomitant downregulation of *Shh* expression specifically in anterior and posterior subdomains of the palatal shelves that correlate with where *Foxf2* is uniquely expressed. Tissue specific inactivation of both *Foxf1* and *Foxf2* in neural crest lineage resulted in specific activation of ectopic *Fgf18* expression throughout the palatal mesenchyme and dramatic loss of *Shh* expression throughout the palatal epithelium. Moreover, addition of exogenous Fgf18 protein to cultured palatal explants inhibits *Shh* expression in the palatal epithelium. Together, these data reveal a novel Shh-Foxf-Fgf18-Shh circuit in the reciprocal epithelial-mesenchymal signaling molecular network controlling palatogenesis. This work was supported by National Institutes of Health National Institute of Dental and Craniofacial Research grant DE013681 to RJ.

POSTER PRESENTATION ABSTRACTS

Poster presentations (in alphabetical order by presenter; presenter is underlined)—* denotes a winner of a graduate student awards for scientific excellence

**** denotes a winner of a post doctoral awards for scientific excellence**

R. Craig Albertson

The genetic architecture and evolvability of phenotypic plasticity: key insights from the cichlid craniofacial skeleton: R. Craig Albertson¹, Moira Concannon², Dina Navon², Jason Wang¹, Ilene Ea¹, Kiran Groveas³, Kevin J. Parsons⁴

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The extent to which the environment shapes the genotype-phenotype map is an important but unresolved question. This issue is especially important with respect to complex traits, which are influenced by a combination of genetic and environmental factors. Quantitative genetic theory and data have demonstrated that the environment can strongly influence the heritability of complex traits, however a strict genetic basis for phenotypic plasticity has remained elusive. Here we explore this question in cichlid fishes. Specifically, we crossed two divergent species to generate an F₃ hybrid mapping population. At early juvenile stages, F₃ families were split and reared for 6 months in alternate foraging environments that imposed distinct kinematic demands on the feeding apparatus. QTL mapping was performed for a variety of traits and we show that the genotype-phenotype map is virtually non-overlapping between treatments, which suggests that cryptic genetic variation is prominent for the craniofacial skeleton. We also detected loci with effects across environments, but which exhibited allele-sensitivity to different foraging conditions, which suggests that plasticity may also be controlled by “master control” loci. The efficiency by which plasticity may evolve is also an open question in the field. Our genetic data provide direct evidence for the evolution of plasticity via genetic assimilation at an important regulatory locus, *ptch1*. Finally, by combining the increased resolution of an F₃ mapping design with

population-level genome scans for divergent SNPs, we offer several environment-specific candidate genes, which may ultimately lead to a better mechanistic understanding of how the environment shapes the craniofacial skeleton. This work was supported by an NSF grant to R.C.A (CAREER IOS-1054909)

Deepti Anand

A new web-based resource SysFACE (Systems tool for craniofacial expression-based gene discovery): Deepti Anand¹, Irfan Saadi² and Salil A. Lachke¹

¹Department of Biological Sciences, Center for Bioinformatics and Computational Biology, University of Delaware, Newark, DE, USA, ²Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS, USA

The NIH FaceBase initiative represents an important resource to expedite craniofacial (CF) gene discovery as it generates comprehensive whole-genome expression datasets using microarrays or Next-Gen RNA-sequencing (RNA-seq) on mouse embryonic CF tissue. However, genome-wide profiling identifies several thousand “expressed” genes, and without any reference for differential analysis, it is a challenge to predict and prioritize the select few that are critical to tissue development or pathogenesis. We recently developed an approach termed *in silico* whole embryonic tissue subtraction to prioritize such important genes based on developmentally-enriched expression ranking. Application of this approach to eye tissue has identified several new genes associated with ocular defects (Lachke, *Science* 2011 331:1571). Here, we expand this approach to develop *SysFACE*, an interactive web resource for (1) efficient prioritization of candidate genes associated with orofacial clefts (OFCs), and (2) derivation of CF tissue gene regulatory networks. Indeed, *SysFACE*-based top 500 ranked genes from the palate, frontonasal, mandible or maxillary tissue datasets are highly enriched in gene ontology categories relevant to CF morphogenesis, such as palate development, cell adhesion, cell proliferation, epithelium development and skeletal system development. In a retrospective analysis, *SysFACE* readily identifies 41 of 45 (91%) OFC-linked genes within the top 5 candidate genes in mean genomic intervals of 13Mb containing ~100 genes. Importantly, *SysFACE* correctly identified the top hits in recent GWAS studies, and has the resolution to identify isoform-specific enrichment from RNA-seq data. Thus, *SysFACE* represents a new resource to expedite CF gene discovery. This work is supported by NIH/NIDCR R03DE024776.

Elizabeth Bhoj

Expanding the SPECCIL mutation phenotypic spectrum to include Teebi Hypertelorism Syndrome: Elizabeth Bhoj¹, Dong Li¹, Margaret Harr¹, Lifang Tan¹, Jodi Hoffman², Hakon Hakonarson¹, Elaine Zackai¹

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Sperm Antigen With Calponin Homology And Coiled-Coil Domains (SPECCIL) encodes a protein involved in microtubule stabilization. Mutations in *SPECCIL* have previously been found in two patients with oblique facial clefts and two families with Optiz G/BBB

Syndrome segregating in an autosomal dominant manner, but no other conditions (Saadi 2011, Kruszka 2015). We performed whole exome sequencing on 24 families with syndromic craniofacial findings. In the two families who had been diagnosed with Teebi hypertelorism syndrome (THS) we found deleterious variants in *SPECCIL*. THS was first described as a new autosomal dominant syndrome in 1987 and subsequently a dozen families have been reported with a range of overlapping phenotypes. We reviewed these 12 families and identified shared features with our patients. 22/22 (100%) had hypertelorism, 16/22 (73%) had umbilical malformations including omphalocele, 5/9 (56%) of males had small scrotum, 6/22 (27%) had natal teeth, 6/22 (27%) had preauricular pits or fistulae, and 3/22 (17%) had cranial suture abnormalities. Phenotypic features in the patients summarized here overlap with previously reported *SPECCIL* patients (Kruszka 2015), all patients had hypertelorism, three patients had umbilical hernias, two patients had craniosynostosis, two had bicornate uterus, and one patient had aortic root dilation. We would like to emphasize that as at least two patients with *SPECCIL* mutations have a dilated aortic root, and a significant percentage have craniosynostosis, therefore these should be screened for in the appropriate clinical setting. As a result, we propose that *SPECCIL* testing is warranted in any patient with a THS diagnosis.

Samantha Brugmann

Understanding the cellular and molecular etiology of craniofacial ciliopathies: Ching-Fang Chang¹, Grethel Millington¹, Samantha Brugmann¹

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Primary cilia are ubiquitous organelles that transduce molecular signals within a cell. Ciliary defects cause a class of human disorders known as ciliopathies. Many ciliopathies are primarily defined by midfacial defects reminiscent of aberrant Sonic Hedgehog (Shh) signaling. Our objective is to understand the role primary cilia play in transducing a Shh signal during craniofacial development. To achieve this objective we examined a Kif3a conditional knockout (Kif3a cKO) model, in which primary cilia are lost on cranial neural crest cells. Kif3a cKO embryos have severe midline widening, micrognathia and aglossia. This phenotype is particularly interesting because whereas midline widening is associated with gain of Shh function, micrognathia and aglossia are associated with loss of Shh function. To determine if these phenotypes were due to dichotomous readouts of Shh pathway activity we examined the production of GLI activator (GLIA) versus GLI repressor (GLIR) isoforms in developing facial prominences. We found ratios of both GLI2 and GLI3 isoforms were disrupted in mutant embryos. To test if disrupted GLI ratios were responsible for both midline widening and micrognathia/aglossia phenotypes we genetically reintroduced only the GLI3R into Kif3a cKO mice. Increasing GLI3R in Kif3a cKOs partially rescued frontonasal widening, but exacerbated micrognathia and aglossia. These data suggest that while midline development is dependent upon GLIR activity, mandibular/glossal development is dependent upon GLIA activity. In sum, our data suggest that each facial prominence is a distinct developmental field requiring cilia-dependent ratio of GLIA/GLIR. This work is supported by NIH/NIDCR R01 DE023804.

Bethany Buckley

A novel ACTB mutation in a patient with Baraitser-Winter cerebrofrontofacial syndrome:
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Baraitser-Winter cerebrofrontofacial syndrome (BWCFF) is a rare developmental disorder characterized by distinct craniofacial features, neuronal migration defects and intellectual disability. Baraitser-Winter cerebrofrontofacial syndrome is a unified term that was recently proposed for the spectrum of disorders caused by mutations in the beta and gamma actin genes (*ACTB* and *ACTG1*). Here we describe a 9 year old male with dysmorphic facial features (bilateral ptosis, epicanthal folds, wide nasal bridge, smooth philtrum, low set partially rotated ears), intellectual disability and developmental delay who was referred to the genetics clinic. Exome sequencing revealed a novel, likely pathogenic variant in the *ACTB* gene. The *ACTB*: c.617G>A variant arose *de novo* in the patient and alters a conserved amino acid residue (p.R206Q). Brain MRI showed mild foreshortening of the corpus callosum with hypoplasia of the rostrum, but no evidence of neuronal migration defects. The patient displayed several additional features not characteristics of BWCFF, including: possible adrenal insufficiency, a distended abdomen, and a diagnosis of autism. This case report expands our knowledge of the *ACTB* phenotypic spectrum and highlights the utility of whole exome sequencing in the diagnosis of rare genetic disorders.

Adrian Danescu

Mutant forms of human WNT5A cause craniofacial defects when expressed in chicken:
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Robinow syndrome is characterized by overall short stature, shortening of the limbs as well as facial abnormalities. The mutations causing Robinow are in genes involved in Wingless signaling including WNT5A, the receptor ROR2 or the adapter protein DVL1. Here we determine the functional impacts of a missense mutation in *WNT5A* on facial development. We created mutations in the human *WNT5A* gene (*WNT5A*^{C83S}) identical to those that cause the dominant form of Robinow Syndrome and inserted the mutant version into an avian retrovirus. These RCAS retroviruses were injected into the mandibular prominence at stage 15. Phenotypes included shortening and deviation of the lower beak towards the injected side especially for the C83S mutation where we noticed the most severe phenotypes (15/15). The earliest morphology changes consisted of a 10% decrease in volume seen at 72h compared to GFP control viruses (p= 0.001). Geometric morphometric analysis did not reveal significant shape changes at 72h. Coincident with the size decrease there was a significant decrease in proliferation on the treated side. Further analysis of cell behaviour and biochemical responses revealed that *WNT5A*^{C83S} is unable to promote JNK signaling as shown with the ATF2 luciferase reporter. In addition cell migration is inhibited in vitro. We conclude that *hWNT5A* mutations shorten the mandible similar to the micrognathia that is

sometimes seen in human Robinow syndrome. Therefore our results contribute to a better understanding of the cellular and molecular basis for the syndrome and of WNT5A function. This work was supported by Canadian Institutes of Health Research doctoral award to AD (RN 0000183232) and CIHR operating grant to JMR (MOP-123536).

Kevin Flaherty

Asynchrony between developmental age and chronological age in mouse embryos: Kevin Flaherty¹, Vivianne Mazzocco¹, Joan Richtsmeier¹

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Embryological research using inbred mouse strains provides much of the basis of our understanding of the genetic and cellular interactions that govern mammalian anatomical development. However, previous research indicates that substantial variation in anatomy and developmental rate exists even within litters of inbred mice. Our ability to understand the genetic and tissue interactions that govern craniofacial development require a precise understanding of the variation present in mouse embryos between chronological time (time since conception) and developmental time (the relatively degree of individual development). Here, we characterize the asynchrony between chronological and developmental age of embryos by analyzing variation in developmental age in inbred mouse embryos. Embryos were collected at 24 hour intervals on embryonic days 12–16 and assigned developmental age estimates based on limb morphology and crown-rump length. Developmental age estimates for specimens were compared with chronological age for both the forelimb and hindlimb in order to characterize the overall pattern of developmental variability during this embryonic period. These results establish an important step towards the ability to precisely characterize developmental ages of mouse embryos, which is itself necessary to determine the order of genetic and tissue interactions that govern craniofacial morphogenetic events during E12–16, such as ossification center formation, bone deposition initiation, and suture formation and fusion. This work is supported by Public Health Service Awards R01-DE018500, ARRA 3R01DE018500-S1, R01-DE022988, P01HD078233; the National Science Foundation BCS-0725227; and the Burroughs Wellcome Collaborative Research Travel Grant (BW Fund 2013).

Everett Hall

Specc11 hypomorphic mouse alleles model human palate and neural tube closure birth defects: Everett Hall¹, Nathan Wilson¹, Diana Acevedo¹, Irfan Saadi¹

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Craniofacial and neural tube (NT) defects constitute more than a third of all congenital malformations. Heterozygous mutations in *SPECC1L* have been identified in patients with atypical and syndromic orofacial clefts, the most common craniofacial birth defect. To study *SPECC1L* function, we have generated severe and hypomorphic mouse alleles of *Specc11* deficiency. The severe allele (RRH) is embryonic lethal at E9.5 with defective NT closure and NCC delamination. In addition, *SPECC1L*-deficient cells show altered adherens

junctions (AJs) and reduced PI3K-AKT signaling, both *in vitro* and *in vivo*. In contrast, the hypomorphic allele (300) results in a 57aa in-frame deletion, and mutants show incompletely penetrant perinatal cleft palate and exencephaly phenotypes (~10% penetrance). In order to increase the penetrance, we crossed the RRH and 300 alleles. *Specc11* 300/RRH compound mutants show markedly increased penetrance (~70%) of palate closure delay. At E14.5, ~50% of the *Specc11* 300/RRH mutants exhibit a partial, V-shaped delay in palate closure, while another ~20% show severe U-shaped cleft with a failure of palatal shelf elevation. We propose a role for SPECC1L in palatal shelf elevation and fusion. Interestingly, we have not yet observed any *Specc11* 300/RRH mutants with exencephaly, which suggests that the 57aa deletion in the 300 allele confers a unique functional consequence that augments occurrence of exencephaly. In conclusion, *Specc11* hypomorphic alleles confirm that moderate reduction in SPECC1L dosage or function resulting from heterozygous human mutations is sufficient to cause perinatal craniofacial malformation, and provide a model to study the underlying pathogenetic mechanisms. This work was supported in part by NIH COBRE (P20 GM104936) and KINBRE (P20 GM103418).

Shawn Hall

Failure of neural tube closure and abnormal craniofacial development in the ENU-induced mouse mutant, *mullet*: [Shawn Hall](#)¹, Kimberly E. Inman¹ and Paul A. Trainor^{1, 2}

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Craniofacial development involves coordinated interaction of germ layer tissues and their derivatives. Neural crest cells, an ectodermal derivative, migrate from the dorsal aspect of the neural tube to the craniofacial region and generate much of the cartilage, bone, and connective tissue of the head and face. To identify novel genes required for craniofacial development in an unbiased manner, we conducted an ENU-induced forward genetic screen in mice. One of the mutant mouse phenotypes we identified was named *mullet*, due to its phenotypic resemblance to a 1980s hairstyle. We have used multiple genomic approaches to map the location of the mutation and have narrowed the region of interest to a 4.5Mb region of chromosome 5. We have begun to characterize *mullet* embryos with respect to neural tube closure and craniofacial development. Our data indicate reduced levels of sonic hedgehog in the ventral portion of the developing neural tube and failure of floor plate induction in *mullet* embryos. Abnormal cranial nerve, dorsal root ganglion, and motor neuron development also occurs. *Mullet* embryos survive until at least embryonic day 18.5 facilitating analyses of neonatal craniofacial development. *Mullet* embryos exhibit abnormal development of maxillary, jaw joint, and middle ear structures together with exencephaly. A preliminary analysis of jaw patterning indicates Bmp4 expression is reduced, which may contribute to improper jaw development. Further analyses will focus on proliferation and apoptosis, dorso-ventral patterning, together with the investigation of actin and cell adhesion dynamics to determine the mechanisms underlying the pathogenesis of neural tube closure and

craniofacial defects in *mullet* embryos. This work was funded by the Stowers Institute for Medical Research.

Greg Holmes

Pathogenesis of the upper airway in craniosynostosis: nasal dysmorphogenesis in Apert syndrome $Fgfr2^{+/S252W}$ and $Fgfr2^{+/P253R}$ mouse models: Courtney O'Rourke¹, Susan Motch Perrine², Claudia Assouline¹, Joan T. Richtsmeier², Ethylin Wang Jabs¹ and Greg Holmes¹

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Apert syndrome is a human genetic disease caused by gain-of-function mutations in *FGFR2*. Craniofacial abnormalities include craniosynostosis and midface hypoplasia, and compromised upper airway function often occurs. The *Fgfr2^{+/S252W}* and *Fgfr2^{+/P253R}* mouse models have been used to study the pathology and abnormal FGFR2 signaling in Apert syndrome. In this study, quantitative analysis of high-resolution magnetic resonance microscopy images revealed the volume of nasopharyngeal passages in P0 *Fgfr2^{+/S252W}* and *Fgfr2^{+/P253R}* mice to be significantly reduced relative to unaffected littermates. We applied histological/immunohistochemical methods and tissue-specific activation of the Apert mutations to characterize changes throughout the nasal passages at the tissue level during embryological development up to birth (P0), and identify their potential etiology. From E16.5 onwards, *Fgfr2^{+/S252W}* cartilage had a significantly greater cross-sectional area throughout the nasal capsule compared to unaffected littermates. By P0, the *Fgfr2^{+/S252W}* nasal passages had a significantly smaller cross-sectional area, although mucosa thickness was decreased. Increased cartilage and decreased nasal space findings were similar in P0 *Fgfr2^{+/P253R}* mice. The cartilage defect was autonomous and independent of suture synostosis, as shown by cartilage-specific *Col2a1-Cre/Fgfr2^{+/S252W}* mutants. Examination of FGFR2 and SOX9 expression at E12.5, or quantification of chondrocyte proliferation and density between E17.5 and P0, did not reveal differences between *Fgfr2^{+/S252W}* and unaffected mice. Our results suggest that Apert FGFR2 mutations increase nasal cartilage by tissue-autonomous mechanisms that result in nasal dysmorphogenesis. This work is supported by NIH R01 DE022988-01 and P0 P01HD078233.

Paul-Pown-Raj Iyyanar*

Hoxa2 regulates osteoblast differentiation in the palatal mesenchyme by modulating Bmp signaling in the developing palate: Paul-Pown-Raj Iyyanar¹ and Adil J. Nazarali¹

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Hoxa2^{-/-} mice exhibit cleft palate although the molecular mechanism underlying the regulation of palate development by *Hoxa2* is largely unknown. Our laboratory has previously shown that *Hoxa2* plays a direct role in secondary palate development regulating cell proliferation. *Hoxa2* is a known inhibitor of osteoblast differentiation in the second

pharyngeal arch, however, its role in the osteoblast differentiation of the developing palate is not known. To investigate this further, primary mouse embryonic palatal mesenchyme (MEPM) cells from wild type and *Hoxa2*^{-/-} E13.5 embryos were subjected to *in vitro* osteoblast differentiation. Increased bone matrix deposition and mineralization was observed in *Hoxa2*^{-/-} MEPM cells *in vitro*. Loss of *Hoxa2* also leads to upregulation of several osteoblast markers including, runt-related transcription factor 2 (Runx2), Alp and *Sp7* in the palatal shelves *in vivo* at E13.5 and E15.5. In addition, in the *Hoxa2*^{-/-} E16.5 embryos, an ectopic expansion of the osteoblast domain (Runx2 and Alp) to the oral half of the palatal mesenchyme was observed. Interestingly, phosphorylated Smad 1/5/8 also exhibited enhanced expression in the oral side of palatal mesenchyme in the *Hoxa2*^{-/-} embryos. Treatment with dorsomorphin, a potent Bmp signaling inhibitor rescued the aberrant cell proliferation and osteoblast differentiation implicating a functional role for canonical Bmp signaling in the *Hoxa2*^{-/-} MEPM cells. Collectively, our data show for the first time that *Hoxa2* functions by regulating osteoblast differentiation of the palatal mesenchyme via Bmp signaling pathway in the developing palate. Supported by a grant from the NSERC to AJN.

Jessica Joganic

Pleiotropic quantitative trait loci (QTL) are a common component of the baboon (genus, *Papio*) cranium's genetic architecture: Jessica L Joganic¹, Katherine E Willmore², Joan T Richtsmeier³, Kenneth M Weiss³, Laura A Cox^{4,5}, John L VandeBerg^{4,5}, Michael C Mahaney⁶, Jeffrey Rogers⁷, and James M Cheverud⁸

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The genetic architecture of a trait determines how genetic variation and environmental factors interact to produce morphological variation on which selection can act. A comprehensive understanding of any complex trait's biology and evolutionary history requires deciphering of its genetic architecture by enumerating the genetic variants affecting the trait and their allelic and interaction effects. Individual genes affecting craniofacial development and variation have previously been identified using model organisms, genome scans, and research with embryos, among other methods. Here, we use quantitative genetics and multipoint linkage analysis to identify quantitative trait loci (QTL) underlying baboon craniofacial variation. Craniometric landmarks were digitized on the crania of baboons (N=985) from the Southwest National Primate Research Center. Sixty Euclidean interlandmark distances (EIDs) were calculated to capture size and shape variation. Oligogenic linkage analysis using a variance components method was conducted in the program SOLAR and QTL for variation in the 60 EIDs were localized within the baboon

genome. We found evidence that many exerted pleiotropic effects on variation in two or more EIDs, reflecting variation in the anterior cranial vault, cranial base, facial breadth, and midfacial/nasal length. These QTL mapped to regions orthologous to ones on human chromosomes 2, 6, 7, 14, 15, 21, and the X. Our findings indicate that genetic modularity is an important component of baboon craniofacial genetic architecture, suggesting that craniofacial traits should not be studied in isolation but in conjunction with traits with which they are genetically correlated. Funding for this research was provided by NSF BCS-0725068 and for baboons by NIH P51 OD11133.

Karyn Jourdeuil

Papillae regeneration reveals insights into epithelial competency for ossicle induction:

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The conjunctival papillae, epithelial thickenings on the surface of the eye, are required for the induction of the underlying neural crest-derived, intramembranous scleral ossicles. These conjunctival papillae are induced beginning at Hamburger and Hamilton (HH) stage 30 following a defined sequence. This induction is completed by HH stage 34 and the conjunctival papillae begin to degenerate at HH stage 37, once they have induced osteogenesis in the underlying mesenchyme. However, little is currently known about the mechanisms underlying the induction and patterning of these papillae. To investigate the involvement of the neural crest cells (NCC) in the establishment of this spatiotemporal pattern, we performed surgical ablations of known signaling centers. We also surgically removed a single papilla in order to investigate the effect on patterning. Papilla removal demonstrated that the conjunctival papillae are capable of regeneration until HH stage 34 and that these regenerated papillae mature faster in order to induce the underlying condensations with only a slight delay. Further research will be required in order to fully understand the mechanisms governing the induction and patterning of the conjunctival papillae. This research was primarily funded by the Natural Sciences and Engineering Research Council of Canada and the Killam Trusts.

Sarah Knox

Parasympathetic nerves and SOX2 control (re)generation of secretory architecture in the salivary gland: Elaine Emmerson^{1#}, Alison May^{1#}, Noel Cruz-Pacheco¹, Carlos O. Lizama², Vagan Mushegyan³, Saunders T. Ching³, Ophir D. Klein³, Ann C. Zovein², William Ryan⁴, Marcus Muench⁵, Sarah M. Knox¹

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contributed equally

Tissue architecture is generated through the coordinated organization of multiple cell lineages. To maintain or reestablish tissue structure during homeostasis and regeneration,

organ compartments must be replenished, each with correctly specified progenitor cells. How this cell specification and replacement is controlled, however, remains poorly understood. Here we demonstrate that parasympathetic nerves in the salivary gland are necessary for specification of the secretory acinar cell lineage and the (re)generation of fetal and adult acini through positive regulation of SOX2. In the absence of cholinergic acetylcholine/muscarinic signaling or after ablation of *Sox2*, the acinar, but not ductal, cell lineage fails to develop, thus depleting the ductal network of functional acini. In the adult organ, resupplying tissue with acetylcholine rescues acinar cell regeneration through expansion and differentiation of SOX2+ acinar progenitors. Thus, we define novel roles of autonomic nerves and SOX2 in regulating cell specification during development and regeneration and propose the use of parasympathetic nerves or mimetics in the regeneration of adult tissues. Our work also highlights the need to explore developmental mechanisms as a means of deciphering and informing regenerative processes for potential therapeutic applications.

Priyanka Kumari

Association of TGFβ3, MSX1, MMP3 and MMP9 with nonsyndromic cleft lip with or without cleft palate in Indian population: Priyanka Kumari¹, Subodh K. Singh² and Rajiva Raman¹

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Several genes have been identified by linkage and expression studies which contribute to the complex etiology of nonsyndromic cleft lip with or without cleft palate (NSCL±P). *TGFβ3*, *MSX1*, *MMP3* and *MMP9* are known to play important role during palate development. Present study evaluates the contribution of these genes to the risk in an Indian cohort. A case-control association study was performed on the coding and promoter region of *TGFβ3*, coding region of *MSX1* & 5A/6A promoter region polymorphism of *MMP3* by sequencing, and -1562C/T of *MMP9* by PCR-RFLP. While coding region of *TGFβ3* did not show any variation there was one variant in the 3'UTR and 3 in the promoter region. Each variant in the promoter occurred within a different transcription factor binding site. Luciferase assay, done to check the effect of these variants on the promoter activity, showed that one of the mutants had a significantly greater activity than the normal allele. Sequencing of *MSX1* gene resulted in 4 known polymorphisms: two each in the coding (one synonymous and one non-synonymous) and intronic regions. Synonymous variant showed association with NSCL±P. Also, 6A allele of *MMP3* 5A/6A polymorphism was found to be associated with NSCL±P whereas no association was seen with respect to *MMP9*. 6A allele of *MMP3* has already been reported to lower its promoter activity. Thus variants of all the studied genes suggest their involvement of *TGFβ3*, *MSX1* and *MMP3* genes in NSCL±P in this population. This work is supported by DBT and CSIR.

Kate M. Lesciotto

Moving towards a mouse model for choanal atresia: Kate M. Lesciotto¹, Susan M. Motch Perrine¹, Kevin Flaherty¹, Kazuhiko Kawasaki¹, Greg Holmes², Etylin Wang Jabs², Joan T. Richtsmeier¹

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Mouse models have proven utility in investigating the pathogenesis of numerous human conditions, including craniosynostosis syndromes. Along with the premature closure of cranial sutures, structural dysmorphologies occur throughout the midface and anterior cranial base and may result in choanal atresia. Choanae permit communication between the posterior nasal cavity and nasopharynx. Osteologically, the human choanae are bordered medially by the vomer, laterally by the medial pterygoid plates of the sphenoid, inferiorly by the perpendicular plates of the palatines, and superiorly by the sphenoidal body. In neonates, blockage of the choanae presents an emergent situation, as infants are obligate nasal breathers. Choanal atresia occurs when bony and/or membranous tissue obliterates the posterior nasal aperture, preventing nasal breathing. However, the study of choanal atresia in murine models for craniosynostosis and other craniofacial conditions raises significant questions. Due to the rostral-caudal elongation of the premaxillary, maxillary, and palatine bones and the soft palate, murine choanae possess different osteological borders than human choanae. Although murine and human choanae have the same physiologic function, inter-specific anatomical differences mean that molecularly-based changes in tissue formation may contribute differently to choanal dysmorphogenesis. In humans, choanal atresia has been attributed to a thickening of the posterior vomer and pterygoid plate medialization. In mice, anatomical separation of these bones makes this an unlikely structural foundation for murine choanal atresia. We review the anatomical correlates of human choanal development in the mouse to further evaluate the usefulness of mice as models for normal and pathological development of these structures. This work is supported by Public Health Service Awards R01-DE018500, ARRA 3R01DE018500-S1, R01-DE022988, P01HD078233; and the National Science Foundation BCS-0725227.

Dong Li

Re-evaluation of a ‘negative’ clinical exome reveals a novel candidate of cleft palate and hypertelorism: Dong Li^{1*}, Elizabeth Bhoj^{1,2*}, Margaret Harr², Elaine Zackai^{2,3}, Hakon Hakonarson^{1,2,3,4}

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Whole exome sequencing (WES) has proven to be a powerful tool for diagnosis in Mendelian disorders. A few large studies on the application of WES have reported a

diagnostic yield of ~25–28%, however the difference in yield in research setting has not been fully investigated. Here we present a family with an apparently autosomal dominant form of cleft palate who tested negative on clinical WES. However, when WES data was obtained from clinical lab and re-analyzed to exclude synonymous variants, variants with minor allele frequency (MAF) greater than 0.5%, and variants previously identified in controls by our in-house exome variant database, we identified a single nucleotide substitution, c.2708C>T:p.(S903F), in *PDGFRA* gene as the most likely candidate. The patient is a 4-month-old male with developmental delay, multiple congenital anomalies (cleft palate, posterior ear pits, tethered chord/meningocele with caudal appendage and left clubbed foot and hypertelorism) and family history is significant for mother born with cleft palate and posterior ear pits and a brother with congenital hyperinsulinism. *PDGFRA* (OMIM 173490), Platelet-derived Growth Factor Receptor alpha, plays a major role in palatogenesis, craniofacial and neural crest development. *Pdgfr*^{-/-} mice have complete cleft palate (CP) that results from an extracellular matrix defect within the cranial neural crest-derived palatal mesenchyme. *PDGFRA* has been associated with isolated CP, however, our family clearly expands the phenotype to include syndromic CP. This report highlights the challenges in recognizing disease variants and that ‘negative’ exome should be worth a second look.

Kerstin Ludwig

Identification of four novel risk loci and functional causative elements in nonsyndromic cleft lip with or without cleft palate: Kerstin U. Ludwig^{1,2}, Anne C. Böhmer^{1,2}, Bhuvu Nagarajan³, Hannah Schuenke^{1,2}, Johanna Klamt^{1,2}, Julian Hecker⁴, Khalid Aldhore⁵, Augusto Rojas-Martinez⁶, Lina Goelz⁷, Alavaro Rada-Iglesias⁸, Heide Fier⁴, Justin Cotney⁹, Markus M. Nöthen^{1,2}, Michael Knapp¹⁰; Benjamin Odermatt³, Elisabeth Mangold¹

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Nonsyndromic cleft lip with or without cleft palate (nsCL/P) is a common congenital malformation with multifactorial etiology. Here, we imputed genome-wide genotype data from a recent meta-analysis (Ludwig et al. 2012, Nat. Genet.) in order to identify novel risk loci. One previously unreported variant showed genome-wide significance (rs6740960 at 2p21). This variant maps 5bp adjacent to a predicted Myc binding site, upstream of *PKDCC*. In a previous report, this gene was downregulated in mice carrying a deletion of the region homologous to the major nsCL/P risk locus at 8q24 (Uslu et al. 2014). Genotyping of 35 selected SNPs with $P < 10^{-03}$ in an independent nsCL/P sample (609 cases, 1,745 controls)

revealed three additional genome-wide significant loci in the combined analysis. We discovered that 30% of the genetic heritability could be attributed to common variants within this dataset. Also, a significant positional overlap of association signals was observed in functional datasets of relevance to craniofacial development. For instance, rs1854110 showed a larger odds ratio than rs8001641, the previous top variant at the 13q31 region. Notably, rs1854110 maps to a putative regulatory enhancer element identified in craniofacial tissue. After cloning this element into a reporter vector based on Tol2/E1b-mediated transgenesis, GFP expression was observed in cephalic regions in zebrafish embryos injected with the plasmid, thus demonstrating the functional effect of the regulatory element. The present study demonstrates that the integration of genomic and functional datasets is a powerful approach to the investigation of craniofacial development and nsCL/P etiology.

Elisabeth Mangold

***GRHL3* mutation screening identifies truncating mutations in patients with apparently nonsyndromic cleft palate:** Elisabeth Mangold¹, Anne C. Böhmer^{1,2}, Pinar Gültepe^{1,2}, Hannah Schuenke^{1,2}, Johanna Klamt^{1,2}, Markus M. Nöthen^{1,2}, Michael Knapp³, Kerstin U. Ludwig^{1,2}

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Recently, grainy head-like 3 (*GRHL3*) was identified as a novel Van der Woude syndrome (VWS) gene. *GRHL3* mutation carriers are more likely to have cleft palate and less likely cleft lip or lip pits than individuals with an *IRF6* mutation. We evaluated apparently nonsyndromic cleft patients for *GRHL3* mutations. We sequenced the complete coding *GRHL3* region in 576 patients with nonsyndromic cleft lip with or without cleft palate (nsCL/P) and 96 patients with nonsyndromic cleft palate only (nsCPO). Observed rare variants were investigated with respect to de novo occurrence. Nineteen rare single-base variants were found, four of them can be regarded as pathogenic: 1 frameshift mutation (present in an affected mother and her child), 3 splice site mutations (two of them de novo in the affected families, one inherited from an unaffected mother to her two affected children). All seven mutation carriers had an apparently nonsyndromic cleft palate only (nsCPO). We re-contacted/re-visited them, and again they denied any VWS typical lip pits. Based on genome-wide imputed nsCL/P GWAS meta-analyses data we investigated whether common variants in the *GRHL3* region are associated with nsCL/P. We tested ~1300 SNPs in the *GRHL3* coding region +/- 200kb for an association. None of the association P-values in that region withstood correction for multiple testing (lowest P-value observed: rs10903069, P=0.013). To identify whether common variants within the *GRHL3* region (coding region, introns, UTR) show evidence for association in aggregate, we performed a gene-based analysis for nsCL/P using VEGAS. No evidence for gene-based association was identified.

David R. Murdock & Tamanna R. Roshan Lal

Solitary Median Maxillary Central Incisor Syndrome: a case report: David R. Murdock¹, Tamanna R. Roshan Lal², Marta W. Biderman¹, Jill A. Fahrner², Joann N. Bodurtha²

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The solitary median maxillary central incisor (SMMCI) syndrome is a rare malformation characterized by the presence of one central incisor as well as defects in other midline structures. SMMCI can occur as an isolated trait but more often is part of the holoprosencephaly (HPE) spectrum. SMMCI is genetically heterogeneous and is associated with mutations in HPE genes including *SHH* and *SIX3*. Here, we report a case of an ex 36+5 week female who presented with respiratory difficulties and dysmorphic features at delivery. Physical examination was positive for midfacial hypoplasia, hypotelorism, mild facial asymmetry, and flattened facial profile with relative microcephaly. A CT of the brain showed a single midline maxillary incisor, with mild to moderate nasal pyriform aperture stenosis. Brain MRI revealed bilateral inferior frontal polymicrogyria but no definite holoprosencephaly. A SNP array showed duplications on 3p25.2 (168kb) and 4q13.3q21.1 (1.1Mb) that were inherited from mother and father, respectively. These regions did not contain any currently known SMMCI or HPE-related genes and were not felt to contribute to the patient's phenotype. Despite not having features of holoprosencephaly, physical examination and imaging in this patient are consistent with SMMCI syndrome. Future plans include molecular testing of genes in the *SHH* pathway as well as careful examination of immediate family members to evaluate for any subtle features of HPE.

Karen Neilson

Candidate co-factors for vertebrate Six family transcription factors are required for otic development: Karen M. Neilson¹, Kristy L. Kenyon², Josephine Stout², Allison Wilcox², Dominique Alfandari³, Sally A. Moody¹

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Several members of the Six family of transcription factors play important roles in vertebrate craniofacial development. It is well documented that Six protein transcriptional activity can be modified by co-factor proteins. Two *Six* genes (*Six1*, *Six5*) and one co-factor gene (*Eya1*) are involved in the human craniofacial syndromes BOS (Branchio-Otic Syndrome) and BOR (Branchio-Otic-Renal Syndrome). However, mutations in *Six* and *Eya* genes only account for about half of the BOS/BOR patients. To discover potential new causative genes, we identified over 30 *Xenopus* genes with high sequence identity to proteins previously shown to interact with the *Drosophila* Six homologue, *SO*, and we determined that many of these are expressed at some stage of craniofacial development (Neilson et al., 2010, Dev Dyn 239: 3446). We now show that 4 of the novel candidate co-factors interact with vertebrate Six1 protein. When their endogenous expression is reduced in the embryo, there is a loss of otic placode genes and an expansion of neural plate genes. When their endogenous expression is increased in the embryo, *Six1* expression is reduced. These results suggest that these *Xenopus* proteins, which have homologues in humans, are previously uncharacterized Six-interacting partners with potential important roles in vertebrate

craniofacial development and congenital syndromes. Funding: NIH R03 HD 055321; NIH R01 DE022065.

Christopher Percival**

QTL analysis of highly varying craniofacial traits in Diversity Outbred mice: Christopher J. Percival¹, Denise K. Liberton², Daniel M. Gatti³, Daniel Pomp⁴, Charles C. Roseman⁵, and Benedikt Hallgrímsson¹

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We use a powerful mouse resource to identify candidate genes associated with normal variation of discrete craniofacial characteristics. The Diversity Outbred (DO) mice are derived from Collaborative Cross (CC) founder strains and each mouse carries a high degree of heterozygosity and a unique combination of alleles. This makes DO mice ideal for high-resolution genetic mapping. We previously identified craniofacial characteristics that vary strongly among the eight CC inbred founder strains, including cranial vault height, jugal bone length, and maxillary palate length. Here, we mapped each of these traits in DO mice (n=563) to identify genomic regions associated with normal craniofacial variation. For each craniofacial trait, we completed a separate analysis using either raw linear distances or residual dimensions after control for overall skull size and allometric variation, because much of the genetic contribution to linear dimensions may be associated with scale, but size corrected variables have become a more common basis for craniofacial morphometric analysis. Generally, similar significant peaks were identified for pairs of raw and residual measures. Cranial vault height was associated with a peak on chromosome 1, where the haplotype of a short vaulted CC parental strain contributed greatly to reduced vault height in some DO mice. Jugal bone length was associated with a peak on chromosome 1 including *Eya1*, previously associated with zygomatic abnormalities, and two peaks on chromosome 17, which include *Runx2* and *Six3*, both previously associated with craniofacial dysmorphology. Funding graciously provided by the NIH (U01DE020054, R01DE019638, R01DE021708, R01GM070683), NSERC (238992-12), and to CJP by the CIHR Training Program in Genetics, Child Development and Health through the Alberta Children's Hospital Research Institute for Child and Maternal Health.

Susan M. Motch Perrine**

Late prenatal effects of the Fgfr3 P244R mutation on murine craniofacial phenotype: Susan M. Motch Perrine¹, Katherine Rhodes¹, Meng Wu², Miao Sun^{3,4}, Ethylin Wang Jabs^{2,4}, Joan T. Richtsmeier¹

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Craniofacial growth is a complex process involving a myriad of essential signaling cascades and growth factors for patterning and development of the head, including fibroblast growth factors (FGFs) and their receptors (FGFRs). The murine P244R gain-of-function mutation of fibroblast growth factor receptor 3 (*FGFR3*) is homologous to the human P250R mutation resulting in Muenke syndrome, a craniosynostosis syndrome characterized by midfacial hypoplasia, abnormalities of the hands and feet, and sensorineural hearing loss. Muenke syndrome accounts for 8% of all craniosynostosis cases (1:30,000 live births) with a highly variable human craniofacial phenotype. We generated a knock-in model of mice heterozygous or homozygous for the *Fgfr3*^{P244R} mutation backcrossed onto C57BL/6 for more than 20 generations, and analyzed craniofacial morphology at embryonic day 17.5 (E17.5) and at birth (P0) relative to their unaffected littermates. Cranial base shortening was shown in *Fgfr3*^{P244R/+} and *Fgfr3*^{P244R/P244R} mice at E17.5 and P0, with *Fgfr3*^{P244R/P244R} mice displaying greater magnitudes of change. No cranial base synchondroses were fused. Nasal and palatal width was increased in all mutant mice at E17.5 and P0. The mandibles were smaller, especially at the posterior portion (coronoid, condyle, and angular regions) in all mutant mice at E17.5 and P0. At E14.5, reduced alkaline phosphatase activity and Runx2 expression were observed in the posterior area of the mandible in *Fgfr3*^{P244R/P244R} embryos. Thus, dysmorphogenesis is most striking in the craniofacial complex where *FGFR3* is known to be expressed in a dosage-dependent manner, with the most severe phenotype seen in *Fgfr3*^{P244R/P244R} mice compared to unaffected littermates. This work is supported by NIH R01 DE022988-01 and P01HD078233.

Kara Powder**

Mutation of a *sox9b* enhancer results in facial malformation; the power of cichlids to identify functionally relevant enhancers: Kara E. Powder¹ and R. Craig Albertson¹

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Mutations in enhancers result in aberrant gene expression and thus disease (including craniofacial disorders) and morphological evolution. Recent studies have experimentally identified >19000 enhancers active during facial or neural crest development. >98% of these have yet to be functionally analyzed due to the challenge of prioritizing and predicting *in vivo* functions. To this end, we utilized the evolutionary system of cichlid fishes, which exhibit extraordinary craniofacial variation that model normal and clinical human variation. Specifically, we mapped enhancers experimentally identified in mammals to the tilapia genome, and cross reference these to both craniofacial QTL and genomic regions that exhibit high levels of genotypic divergence among phenotypically divergent cichlid species. This approach (1) prioritizes enhancers in an efficient and unbiased way from thousands to tens, (2) identifies those most likely to regulate variation in craniofacial morphology, and (3) links enhancers to specific phenotypes without assumed action on neighboring genes. Using this method, we prioritized 44 (of 7282 enhancers; Attanasio, et al. 2013) for future functional studies, including a putative *sox9b* enhancer. Mutations in human *SOX9*

enhancers are associated with Pierre Robin Syndrome, which includes micrognathia. We show that disruption of the *sox9b* enhancer using CRISPR in zebrafish results in the highly specific loss of ceratobranchial cartilages and mild mandible malformation; these phenotypes are consistent with *sox9b* mutants, which lack chondrogenesis. Combining bioinformatic prioritization using cichlid data and gene editing via CRISPR offers a powerful approach to study the function of enhancers. Supported by NIH/NIDCR 1F32DE023707 (KEP); NSF CAREER IOS-1054909 (RCA).

Ryan Roberts*

Fgfr2 regulates formation of tendon/ligament-to-cartilage attachment units in the craniofacial complex: [Ryan Roberts](#)¹ and Amy Merrill^{1,2}

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Fibroblast Growth Factor (FGF) signaling plays a critical role in skeletal development, as mutations in *Fibroblast Growth Factor Receptor 2* (FGFR2) manifest with at least 10 distinct skeletal birth defects. Loss of function mutations in *FGFR2* cause Lacrimoauriculodentodigital (LADD) Syndrome, which is characterized by microretrognathia; a posterior shortening of the jaw, as well as a number of other craniofacial and skeletal abnormalities. We hypothesize that the posterior jaw shortening in LADD Syndrome indicates a role for FGFR2 in the development of the jaw processes. To test this hypothesis, we utilized a conditional knockout mouse in which *Fgfr2* is ablated within the Wnt1 expressing neural crest cells that provide the skeletal precursors for the jaw. We found that *Fgfr2^{flx/flx}; Wnt1-Cre* mice have jaw defects at sites of joint integration and at the tendon/ligament-to-bone attachment units on the condyle, angular process and ramus. Histological and molecular markers indicate that at these regions, endochondral-like bone has replaced the developing enthesis, which has been shown to differentiate into chondrocytes, tenocytes and ligamentocytes. Additionally, reporter mouse genetics suggest that the change in cell fate is autonomous to the enthesis progenitor cells and not caused by the ablation of *Fgfr2* in the underlying bone. Altogether, this suggests a role for Fgfr2 in cell fate determination of the enthesis progenitor cells in the jaw. This work is supported by NIH/NIDCR Training Grant #T90DE21982; NIH/NIDCR R01 DE025222-01 Diversity Supplement; James H. Zumberge USC Faculty Research and Innovation Fund; Basil O'Connor Starter Scholar Research Award #5-FY12-166; March of Dimes; NIDCR award 5 P30 DE020750-02 to Dr. Yang Chai; NIH/NIDCR R01 DE025222-01.

Lisa Sandell

Retinoic acid regulates embryonic development of mammalian submandibular salivary glands: [Lisa L. Sandell](#)

University of Louisville School of Dentistry

Embryonic development of the head and face is largely orchestrated by cell and tissue interactions mediated by molecular signals. Knowledge of the identity of such signals, as

well as the identity of tissues that generate them and respond to them, is important for understanding the developmental formation of craniofacial structures and organs. Here we demonstrate that morphogenesis of mammalian submandibular salivary glands (SMG) is regulated by Retinoic Acid (RA), the active metabolite of Vitamin A. By examining mouse embryos with a mutation in the gene encoding retinol dehydrogenase 10 (RDH10), which are deficient for Vitamin A metabolism and RA production, we show that RDH10-mediated metabolism of Vitamin A is necessary for SMG development, and that RA regulates SMG morphogenesis in a dose-dependent manner. Additionally, we find that active RA signaling occurs in SMG tissues, arising earlier than any other known marker of SMG development and persisting throughout gland morphogenesis. At the initial bud stage of development, we observe a reciprocal tissue distribution of RA production and RA signaling, with gland mesenchyme expressing the *Rdh10* that is needed for RA production, while gland epithelium experiences RA signaling activity. Finally, we demonstrate that active RA signaling occurs in glands cultured *ex vivo*, and inhibition of RA signaling in cultured glands by treatment with a chemical inhibitor blocks growth and branching morphogenesis. Together these data identify RA as a key signaling molecule that directly regulates organogenesis of mammalian SMG. Funding Acknowledgement: This work was supported by funding from P20 RR017702 and GM103507.

Richard Schneider

Regulation of jaw size during development, disease, and evolution: Richard A. Schneider

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The remarkable success of vertebrates is due in large part to the ability of the jaw to vary in size during evolution. A wide range of genetic and embryological studies have shown that the establishment of jaw size is a complex process involving numerous gene regulatory networks, reciprocal signaling interactions, and hierarchical levels of control. Yet what remains unclear are precise determinants of species-specific jaw size that presumably operate during the allocation, proliferation, differentiation, and growth of jaw progenitors. To address this issue, we use Japanese quail, which have short jaws, and white Pekin duck, which have relatively long jaws. We transplant neural crest mesenchyme (NCM), which are the progenitor cells destined to form the jaw skeleton, between quail and duck embryos. Resulting chimeras are challenged to integrate two distinct morphogenetic programs and enable us to pinpoint mechanisms underlying the establishment of species-specific jaw size. We have found that NCM employs a variety of precise mechanisms to govern jaw size through three principal phases of development. First, during the migration and allocation of NCM, quail and duck have distinct numbers of progenitors destined to form the jaw skeleton; second, when these populations proliferate and expand, there is species-specific regulation of, and response to, multiple signaling pathways; and third, as these progenitors differentiate into the jaw skeleton, they execute autonomous molecular and cellular programs for bone deposition and resorption intrinsic to each species. Thus, NCM deploy sequential but distinct molecular and cellular mechanisms that control jaw size during development, disease, and evolution. Funded, in part, by NIDCR R01 DE016402

Ankita Shah

Functional role of Annexin A6 during cranial gangliogenesis: Ankita Shah¹ and Lisa A. Taneyhill¹

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The cranial sensory ganglia are created from two migratory cell populations, neural crest cells (NCCs) and placode cells (PCs). Defects in the migration and interaction of these cells can cause abnormalities in craniofacial development and in the formation of the sensory nervous system. The molecules that govern NCC-PC interactions during ganglia assembly, however, are not well characterized. To this end, we are using the developing trigeminal and epibranchial ganglia in the chick embryo to elucidate mechanisms underlying NCC-PC interactions. The Annexin protein superfamily is crucial for sensory ganglia development, a process that likely requires actin cytoskeletal remodeling to mediate cell motility and intercellular interactions. Our lab identified a function for chick Annexin A6 in modulating NCC migration, but a later role for Annexin A6 in cranial ganglia assembly has not been investigated. We hypothesize that Annexin A6 acts as a core cytoskeletal scaffold in PCs to facilitate ganglia formation. In support of this, we show that PCs, not NCCs, express Annexin A6 during ganglia assembly, and that Annexin A6 possesses a functional F-actin binding site *in vitro*. Finally, depletion of Annexin A6 from PCs perturbs PC polarity but does not alter PC position within the ganglionic anlage or disturb surrounding NCC corridors. Future studies will focus on understanding how the association of Annexin A6 with the actin cytoskeleton impacts PC morphology and overall ganglia assembly. Our results will provide insight into the mechanisms that underscore cellular movements essential for cranial gangliogenesis and in other tissues generated from heterotypic cell types. This work is supported by a grant to LAT (NIH R01DE024217).

Richard Sherwood

Craniofacial growth prediction: Richard J. Sherwood^{1,2}, Sheldon Baumrind³, Mark G. Hans², James A. McNamara Jr.⁴, Kieran P. McNulty⁵, Manish Valiathan², Heesoo Oh³

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Effective growth modification interventions in pediatric craniofacial treatment require detailed knowledge of the remaining facial growth and the timing of periods of differential growth velocity. Further, understanding the proportion and pattern of growth remaining after completion of treatment is important for prediction of post-treatment rebound. Proper treatment timing relative to expected maxillary and mandibular skeletal growth rates impacts the success of various treatments by providing improved results in less time. We present the first stage in our ongoing craniofacial research program which will include development of individualized prognostic tools for prediction of remaining craniofacial growth via a web-based presentation of results for a range of craniofacial traits. We have recently begun

assessment of standard cephalometric points for over 30,000 mixed longitudinal cephalographs in multiple North American populations. Initial work for this project used serial data available in the Fels Longitudinal Study. We demonstrate this approach by estimating growth parameters for the cephalometric measures (e.g., Ar-Me; PNS-PtA) in both sexes using polynomial growth curves. Parameters include age at onset of adolescent growth, peak growth velocity and cessation of adolescent growth; duration of time between ages, amount of growth between ages, and magnitude of peak growth velocity. For boys, across facial traits, the population-average ages of onset of adolescent growth range from 8.28 to 9.38 years, the ages of peak growth velocity range from 13.02 to 14.79 years, and the ages of cessation of adolescent growth range from 18.54 to 20.99 years. For girls, these population-average ages are earlier for each trait. Funding: NIH/NIDCR R03-DE021435; NIH/NIDCR R01 DE024732; NIH/NIDCR R01 DE016692.

Rolf Stottmann

Gene discovery in mammalian craniofacial development and disease: the primary cilium and beyond: Chelsea Menke¹, Andrew DiStasio¹, Laura Runck¹, Marshall Lukacs¹, Rolf Stottmann^{1,2}

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We have used a forward genetic approach to identify novel genes in the mouse which are required for normal craniofacial development. ENU mutagenesis results in single base pair changes in the genome and is an efficient tool to create mouse alleles which are good models of human disease. Exome sequencing approaches have greatly enhanced our abilities to clone mutations from the ENU mutagenesis project. We will summarize our findings to date, including the identification of mutations in multiple genes not previously discussed in the craniofacial literature. One of the more intriguing alleles we have identified and begun to characterize is *alien*, a null allele of *Ttc21b* (*tetratricopeptide repeat domain-containing protein 21B*). *Ttc21b* is required for normal retrograde intraflagellar transport in the primary cilium and is the most often mutation gene in human ciliopathy studies. *Ttc21b^{aln/aln}* homozygous null mutants have profound craniofacial defects which we have begun to explore in some detail. We have utilized a conditional allele of *Ttc21b* to identify spatiotemporal requirements during anterior embryonic development. We have identified biochemical and genetic interactions between *Ttc21b* and *Gli3*, as well as *Ttc21b* and *Shh*. We are pursuing these findings further with a genetic approach to identify other members of the *Ttc21b* interactome. Funding : Cincinnati Children's Hospital Research Foundation, NIH (NIGMS).

Heather Szabo-Rogers

The role of Prickle1 in cranial base development: Yong Wan¹, Brian Cusack¹, Bansari Amin¹, Heather Szabo-Rogers¹

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Normal development of the face and skull requires the coordinated growth and expansion of the cranial base. The role of the cranial base in proximal distal growth of the face is often overlooked. We have identified Beetlejuice, an ENU (N-ethyl-N-nitrosourea)-mutagenized mouse line – that develops defects in both the cranial base and face. The Beetlejuice line has a damaging mutation in the core planar cell polarity protein, Prickle1. During early stages of craniofacial development, Prickle1 protein and mRNA is found in the mesenchyme of the maxillary, and mandibular arches, as well as the cranial base anlagen. The Beetlejuice mutants die perinatally as a result of cleft palate and heart defects, and also have a shortened head and rounded skull. At birth, the craniofacial skeleton has several defects including smaller maxilla, mandible, nasal and frontal bones and some sutures develop ectopic bone. Intriguingly, we have found that the anterior cranial base is enlarged in the medio-lateral axis with a concomitant reduction in the anterior-posterior axis. The craniofacial defects found in the Beetlejuice mutants are confined to neural crest-derived tissues. Based on the expression of Prickle1, and the Beetlejuice skeletal phenotype, we hypothesize that Prickle1 is required for neural crest cell migration and differentiation. We are currently testing whether the neural crest cells have defects in migration, or survival. The Beetlejuice line is an important new model for understanding how planar cell polarity pathway regulates craniofacial development.

Karla Terrazas*

Dissecting the roles of Tcof1, Polr1c and Polr1d in ribosome biogenesis, craniofacial development, and disease: Karla Terrazas¹, Annita Achilleos¹, George Bugarinovic¹, and Paul Trainor¹

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Neural crest cells (NCC) are a transient, multipotent, migratory population that is unique to vertebrates and gives rise to an array of tissues including craniofacial cartilage and bone, and the peripheral nervous system. Many birth defects, termed neurocristopathies, are attributed to deficiencies in NCC induction, migration, proliferation, and differentiation. To better treat and prevent neurocristopathies it is critical to understand the processes that regulate each phase of NCC development. One such process that is critical for cell survival, through its regulation of cell growth and proliferation, is ribosome biogenesis. Although this process is believed to function globally, tissue specific congenital anomalies can occur as a result of perturbations in ribosome biogenesis. These conditions are termed ribosomopathies and include Treacher Collins syndrome (TCS), which is characterized by craniofacial anomalies including hypoplasia of facial bones and cleft palate. Mutations in 3 genes have been identified in TCS patients: *Tcof1*, *Polr1c* and *Polr1d*, all of which are involved in ribosomal DNA (rDNA) transcription. TCS arises through defects in NCC development and we are exploring the roles that *Tcof1*, *Polr1c* and *Polr1d* play in NCC development and survival as well as in ribosome biogenesis in the pathogenesis of TCS and other ribosomopathies. We are also investigating the potential for genetic interactions between *Tcof1*, *Polr1c* and *Polr1d* and the tissue specific requirements for ribosome biogenesis during embryogenesis. Our results will aid in understanding the roles that ribosome biogenesis plays in NCC development and in the pathogenesis of congenital anomalies. This work is supported by the

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Karin Weiss

Adult patients with holoprosencephaly; the face may not predict the brain: Karin Weiss¹, Maria Guillen Sacoto¹, Paul Kruszka¹, Maximilian Muenke¹

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Holoprosencephaly (HPE) encompasses a continuum of brain malformations that include (in order of decreasing severity); alobar, semilobar, lobar, middle interhemispheric variant and septopreoptic type. The most common facial dysmorphisms include hypotelorism, midface hypoplasia, cleft lip and/or palate, and a single maxillary central incisor. Traditionally, it was thought that in HPE “the face predicts the brain”: in other words, more severe craniofacial anomalies correlate with more severe neuroanatomic findings and survival. It has already been reported that this correlation may not occur as frequently in patients with *ZIC2* mutations. During 2015 we evaluated a unique group of adult patients with HPE, ascertained through the international registry of HPE. There were three males and six females, aged 16–33 years (mean 21). The MRI scans showed semilobar HPE in six of the patients, lobar HPE in two and the septopreoptic type in one. Seven patients had only subtle dysmorphic features and none of the hallmark facial features of HPE. Two patients had at least one classical feature (midface hypoplasia, hypotelorism, single central incisor), but their MRI scans showed milder abnormalities. The neuropsychological development correlated with the brain anatomy and was variable: two individuals walk independently and communicate verbally, two are non-ambulatory and communicate verbally and 5 are non-verbal and non-ambulatory. The patients we evaluated had negative testing for the 4 most common genes associated with HPE (*SHH*, *ZIC2*, *SIX3*, *TGIF*) and we speculate that patients who survive into adulthood have a different etiology.

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