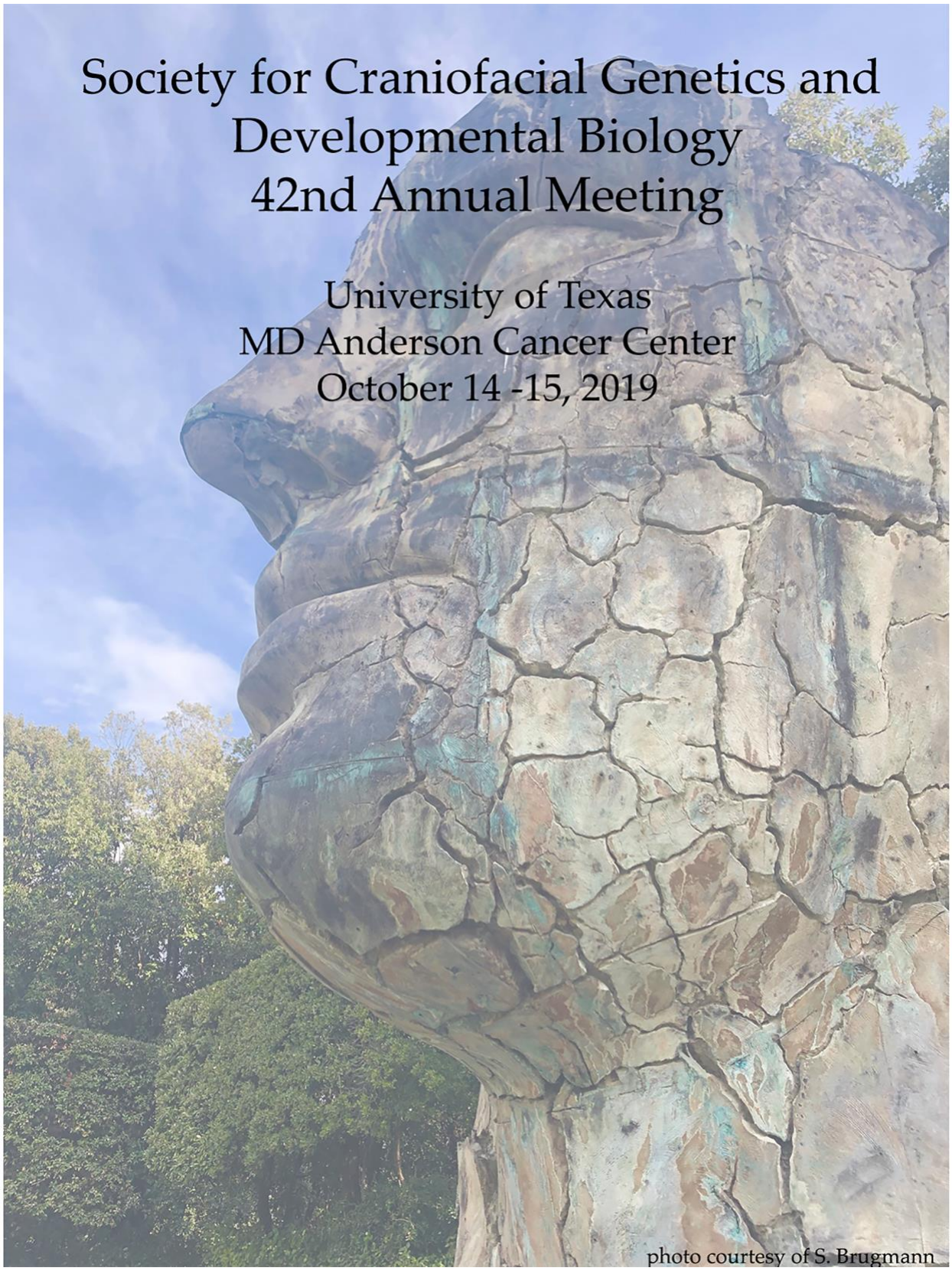
Society for Craniofacial Genetics and Developmental Biology
42nd Annual Meeting

University of Texas
MD Anderson Cancer Center
October 14 -15, 2019



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The Society for Craniofacial Genetics and Developmental Biology

Harassment Statement

The SCGDB is dedicated to providing a meeting environment that is grounded in dignity and respect for all program participants, regardless of sex, gender identity and expression, sexual orientation, disability, physical appearance, ethnicity, religion, or age. Program participants, including the Officers, Executive Board, other volunteers, members, event attendees, consultants, vendors and any others attending the event, are expected to conduct themselves in a professional manner and to treat each other with respect. In addition, the SCGDB will not tolerate acts or threats of violence against program participants. Should an incident occur while at an SCGDB-sponsored meeting, the SCGDB Officers, Executive Board, and session or workshop chairs should be considered safe authorities with whom incidents can be discussed.

The Society of Craniofacial Genetics and Developmental Biology: a brief history

By: Geoffrey H. Sperber
School of Dentistry, University of Alberta, Edmonton, AB, Canada
With: Paul Trainor, Stowers Institute for Medical Research, Kansas City, MO

The Society of Craniofacial Genetics (SCG) was founded in 1975 under first President, David Bixler (1929-2005), with the motto :"Educatio, Investigatio, Communicatio". The SCG held its inaugural meeting in 1975 in Baltimore, MD and initially published a Newsletter: "*The Locus*". Subsequent meeting reports were recorded in the SCG's official journal: "*Journal of Craniofacial Genetics and Developmental Biology*", published by Blackwell, which ceased publication in 2000.

In the early years of SCG, annual meetings were held in conjunction with March of Dimes Birth Defects meetings. Later, SCG began to host their meetings immediately preceding the American Society of Human Genetics (ASHG) annual meetings. Meetings were held successively in various cities in the USA, Mexico and Canada. In 2012 the Society changed its name from the Society for Craniofacial Genetics to the Society for Craniofacial Genetics and Developmental Biology (SCGDB). The addition of Developmental Biology reflected the increased diversity of professional interests among members, especially in developmental biology and the intersection of development, genetics, evolution and disease. This year's meeting in Houston marks the 44th year of the Society's existence.

In response to a proposal by Paul Trainor (President 2015-2016), the attendees in 2015 voted to alternate the timing of the of the SCGDB annual meeting between the ASHG and the Society for Developmental Biology (SDB) or other meetings and craniofacial centers as desired. This reflected the developmental and evolutionary biology interests of its members in addition to genetics and disease. Accordingly, the 2015 meeting saw the expansion of the meeting from one day to an afternoon/evening plus a full day. This was designed to accommodate the addition of a dedicated clinical session involving surgeons and clinical geneticists, and to provide more opportunities and time for oral and poster presentations, especially from junior investigators.

The 2016 and 2017 SCGDB annual meetings were held in Boston and Minneapolis respectively, in conjunction with the Society of Developmental Biology (SDB)'s 75th and 76th annual meetings. The SCGDB's 2016 meeting was combined not only with the

SDB's 75th annual meeting but also with the International Society of Differentiation's 19th International Conference in Boston, MA, August 4-8, 2016. The expanded meetings added clinical sessions to include patient advocates in addition to surgeons and clinical geneticists. An extensive exhibition by publishers and research centers highlighted this meeting (Taneyhill et al., 2016). The 2018 and 2019 SCGDB annual meetings coincided with ASHG in La Jolla and Houston, respectively. Reports of the abstracts of the papers presented at these meetings have been published in the *American Journal of Medical Genetics* since 2011 (see References below).

The 2019 annual meeting marks the introduction of The David Bixler and the Marylou Buyse Excellence in Craniofacial Research awards, sponsored by *Developmental Dynamics*. These research awards were proposed by the SCGDB Advisory committee (Paul Trainor – past president, and Scott Lozanoff – past secretary/treasurer) to recognize the achievements of SCGDB members, and to also promote the field of craniofacial biology. The David Bixler and Marylou Buyse awards celebrate meritorious achievements in craniofacial biology by senior career and mid-career SCGDB members, and are respectively named after the first SCGDB President, and first female SCGDB President. The first two awardees are Joan Richtsmeier and Amy Merrill-Brugger.

The 2019 annual meeting also marks the establishment of SCGDB's Distinguished Service Award. In parallel with the Excellence in Craniofacial Research Awards, the current SCGDB Executive Board, led by President Sally Moody, recognized the importance of appreciating the dedicated service of members to SCGDB and created a Distinguished Service Award. Geoffrey Sperber who served as the Secretary/Treasurer of the SCGDB from 2004 to 2011 is the first awardee.

Each of SCGDB's awardees are being recognized at the annual meeting through an oral presentation and receipt of a commemorative plaque.

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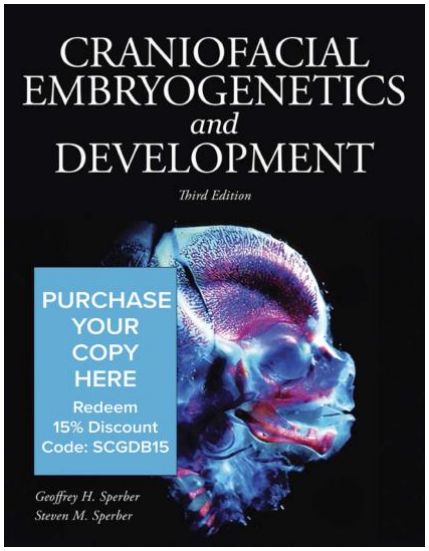

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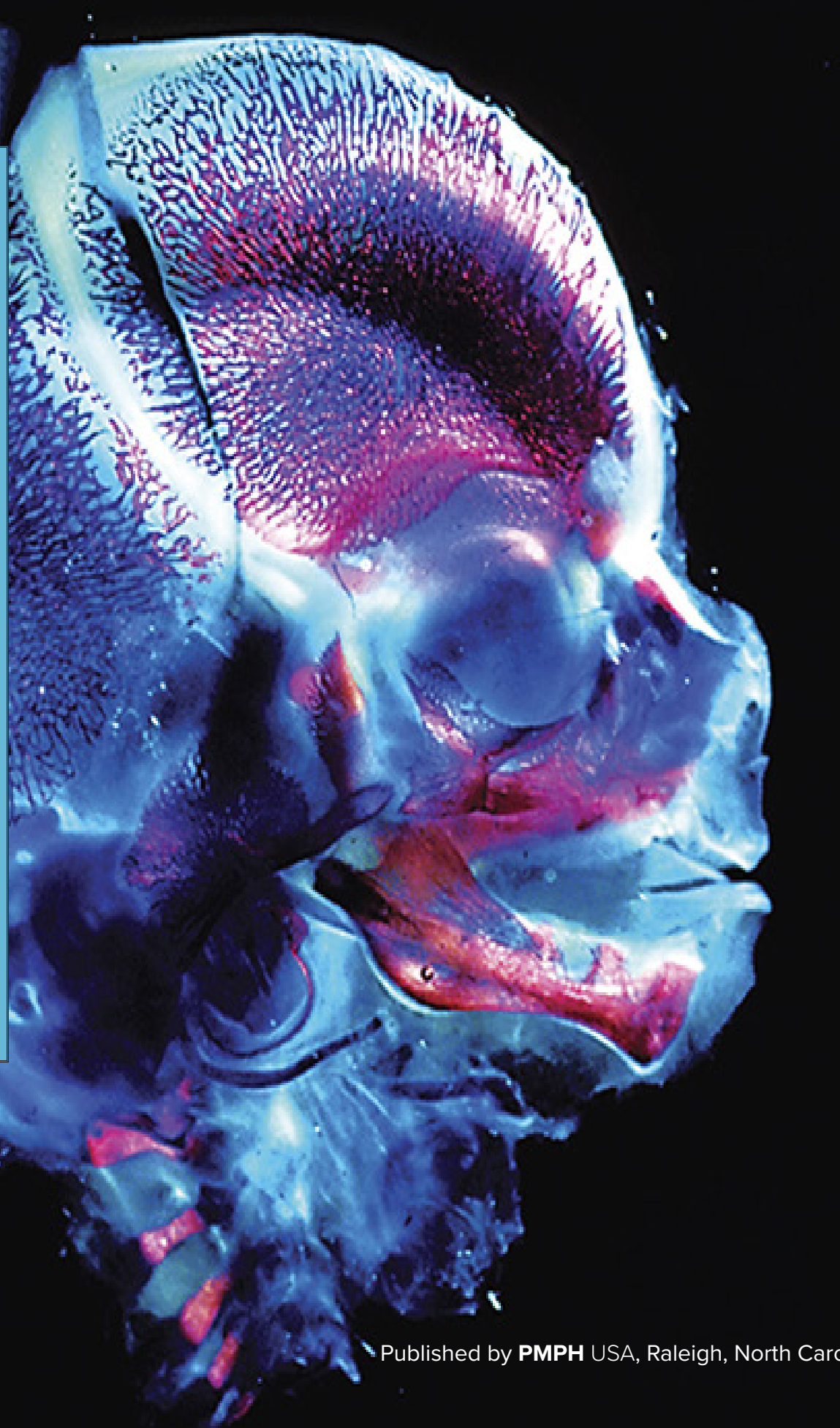

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Geoffrey H. Sperber
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Society for Craniofacial Genetics & Developmental Biology
42nd Annual Meeting

October 14-15th, 2019
MD Anderson Cancer Research Center
University of Texas, Houston, Texas

PROGRAM

Monday, October 14th, 2019
Main Building, Hickey Auditorium (R11.1400), 1515 Holcombe Blvd

Career Development Workshop (10:00 – 11:30 AM)

10:00 – 10:30: What do I look for in a mentor?

Leaders: Timothy Cox, University of Missouri-Kansas City & Jean-Pierre Saint-Jeannet, New York University

10:30 – 11:00: How do I deal with manuscript/grant rejection?

Leaders: Sally Moody, George Washington University & Paul Trainor, Stowers Institute for Medical Research

11:00 – 11:30: Harassment, unconscious bias and bullying – how do we deal with it?

Leaders: Lisa Taneyhill, University of Maryland, College Park & David Clouthier, University of Colorado Anschutz Medical Campus

Lunch on your own

Special Awards Session (1:00 – 3:00 PM)

Session Chair: Sally A. Moody, George Washington University

1:00 – 1:30: **Lifetime Achievement Award**

Geoffrey H. Sperber, University of Alberta
"Reflections on the SCGDB and "Craniofacial Embryogenetics and Development"

1:30 – 2:15: **David Bixler Excellence in Craniofacial Research Award**

Joan Richtsmeier, Pennsylvania State University
"Cranial cartilage and bone formation in development and disease"

2:15 – 3:00: **Marylou Buyse Excellence in Craniofacial Research Award**

Amy Merrill-Brugger, University of Southern California
"Developmental control of cell fate choice: insights from rare craniofacial disorders"

Coffee break

Molecular Regulation of Craniofacial Development (3:30 – 5:00 PM)
Session Chair: David Clouthier, University of Colorado Anschutz Medical Campus

3:30 – 4:00: **Julia Boughner**, University of Saskatchewan
"New genes underpinning evo-devo integration of vertebrate dentitions and jaws"

4:00 – 4:15: **Rolf Stottmann**, Cincinnati Children's Hospital Medical Center
"The primary cilium gene Ttc21b modulates craniofacial development as a crucial ciliopathy gene"

4:15 – 4:30: **Rachel Keuls**, Baylor College of Medicine
"Neural crest development is temporally regulated by miR-302

4:30 – 4:45: **Andre L. P. Tavares**, George Washington University
"Identification of novel candidate genes associated with SIX1 and Branchio-oto-renal syndrome"

4:45 – 5:00: **Lindsey Barske**, Cincinnati Children's Hospital Medical Center
"Evolution of vertebrate gill covers through shifts in an ancient gnathostome Pou3f3 enhancer"

Poster Session and Reception (5:00 - 8:00 PM)
Light refreshments

Tuesday October 15th, 2019
Duncan Cancer Prevention Building, 8th floor, rooms 5-8

Cell Biology of Craniofacial Development (8:30 – 10:00 AM)
Session Chair: Lisa Taneyhill, University of Maryland College Park

8:30 – 9:00: **Amanda Dickinson**, Virginia Commonwealth University
"Using frog faces to dissect mechanisms underlying human orofacial defects"

9:00 – 9:15: **Annita Achilleos**, Baylor College of Medicine
"Ronin (Thap11) regulates neural crest and craniofacial development via the vitamin B12 pathway"

9:15 – 9:30: **Katherine Fantauzzo**, University of Colorado Anschutz Medical Campus
"Srsf3-mediated alternative RNA splicing downstream of PDGFRα signaling in the palatal mesenchyme"

9:30 – 9:45: **Nadège Gouignard**, New York University
"Paracrine activation of neural crest EMT genes by placodal MMP28"

9:45 – 10:00: **Junichi Iwata**, University of Texas Health Science Center at Houston
"Cholesterol-dependent primary cilium formation is crucial for osteogenesis"

Coffee break

Translational Craniofacial Biology (10:30 AM – 12:00 Noon)
Session Chair: Timothy Cox, University of Missouri-Kansas City

10:30 – 11:00: **Steve Murray**, The Jackson Laboratory
"Insights from large-scale discovery of mammalian essential genes"

11:00 – 11:15: **Soma Dash**, Stowers Institute for Medical Research
"A novel role for the mediator complex protein Med23 in craniofacial development and in the pathogenesis of Pierre-Robin Sequence"

11:15 – 11:30: **Courtney Willett**, Emory University
"A novel 3MB deletion on 6p24 excluding *TFAP2A* results in a mild phenotype in a multiplex family with orofacial clefting"

11:30 – 11:45: **Bhavna Tandon**, University of Texas McGovern Medical School
"Role of MMP2 in early craniofacial development in zebrafish"

11:45 – 12:00: **Ela W. Knapik**, Vanderbilt University Medical Center
" Integrated analysis of *RIC1* mutations and phenome in a Biobank identifies novel craniofacial syndrome and collagen trafficking mechanisms"

SCGDB Members meeting (12:00 – 1:30 PM) Boxed lunch provided

Signaling during Craniofacial Development (1:30 – 3:00 PM)
Session Chair: Jean-Pierre Saint-Jeannet, New York University

1:30 – 2:00: **Plenary talk**: **Andrew Groves**, Professor, Baylor College of Medicine
"Developmental genetic regulation of the mammalian inner ear"

2:00 – 2:15: **Jaeho Yoon**, National Cancer Institute-Frederick
"TBC1d24-ephrinB2 interaction regulates contact inhibition of locomotion in neural crest cell migration"

2:15 – 2:30: **Xiaolei Zhao**, University of Texas McGovern Medical School
"Hippo pathway interacts with Wnt pathway to control the neural crest cells"

2:30 – 2:45: **Ariadne Letra**, University of Texas Health Science Center at Houston
"Functional effects of *WNT10A* rare variants identified in individuals with isolated tooth agenesis"

genesis, The Journal of Genetics and Developmental Biology **Trainee Speaker Prize**
2:45 PM

Adjourn - 2:50 PM

Members' meeting agenda

12:00-12:10: President's Comments (Moody)

- Program organizers
- Next year's meeting
- Society Awards
- Next year's elections

12:10-12:20: Treasurer's report (Saint-Jeannet)

12:20-12:35: Election of Secretary (Taneyhill)

12:35-12:50: Awards (Clouthier)

- Postdoc poster awards
- Graduate student poster awards

12:50-1:30: Discussion and vote on affiliation with AAA (Trainor)

SCGDB affiliation with AAA

Under previous leadership, the SCGDB has grown. We now have a small endowment, which we can use to begin to provide long-term financial stability for the society and benefits for our members. While this is fantastic news for our society, it creates logistical problems in terms of stewardship of finances, fiduciary responsibilities and legal issues for individuals serving on the Board of Directors, all of whom are volunteers. More specifically, the Board of Directors incur personal legal responsibility for management of SCGDB funds including taxes since SCGDB is a designated not-for-profit entity. The Board of Directors can also be held responsible for actions undertaken by the SCGDB and its members at SCGDB events. Societies typically provide Directors Insurance to mitigate the risks associated with these issues, but this is very costly for small societies such as SCGDB. In addition, SCGDB does not yet have enough funds to invest independently. To solve both of these problems, SCGDB has explored an affiliation agreement with the American Association for Anatomy (AAA). Importantly, affiliation with AAA provides additional opportunities for SCGDB and its members.

- SCGDB would become an affiliated "section" of AAA.
- The SCGDB board members would be covered by AAA Directors Insurance for as long as the affiliation agreement remains in place.
- SCGDB funds will remain separate from AAA funds and remain exclusively for the use of SCGDB.
- AAA will provide SCGDB with taxation filing advice and website support, as well as logistical support for the SCGDB annual meeting in terms of registration, abstract submission, badge printing and program preparation.
- The initial term of the affiliation agreement is intended to be 5 years, with evaluation of the success of the affiliation after 1, 3 and 5 years. If the affiliation is deemed not to be working, SCGDB and AAA each have the opportunity to end the affiliation.

Affiliation with AAA logistics:

- All SCGDB members would initially join AAA as the first step, and then for an additional nominal amount ($5), elect to become a member of SCGDB
  Current SCGDB membership: Regular: $75, Post-doc $40, Grad student: $25
  Current AAA membership: Regular: $130, Post-doc: $45, Grad student: $30

- The additional $5 SCGDB membership fee provides voting rights for SCGDB members in SCGDB elections, which regular AAA members will not have.

- SCGDB membership provides eligibility for SCGDB Excellence in Craniofacial Research, Service, travel and other SCGDB sponsored awards.

- SCGDB will continue to elect its officers at each annual meeting from SCGDB members.

- To be covered by AAA Directors Insurance, AAA requires that 50% of the SCGDB Board be AAA members.

Benefits of Partnering with AAA for SCGDB and SCGDB members:

- SCGDB maintains its independence but with support from AAA can grow.

- The leadership structure of SCGDB remains the same, and the character of its annual meeting in conjunction with ASHG, SDB or a specialized craniofacial center with a local organizer remains the same.

- Creates an international platform for craniofacial biology and broader exposure for SCGDB

- SCGDB/Craniofacial Biology presence at the AAA/EB annual meeting (6000+ attendees)

- SCGDB members will be eligible for conference travel awards and trainee poster awards at the AAA/EB annual conference (usually held in April).

- SCGDB members will be eligible for AAA Visiting Scientist awards, Post-doc Fellowships, Early Career Stage (Assistant Professor) awards, Fellows awards, Scientific Research awards, Distinguished Educator awards, Leadership/Service awards, FGAP (research funding) awards, Outreach meeting grants.

- AAA may provide funding to support the SCGDB annual meeting. As an act of good faith support, AAA provided $5000 for this meeting, and there is a possibility our annual meeting could be co-branded as a AAA regional meeting (with funding support from AAA), which could further help the meeting and SCGDB community to continue to grow.

Special Awards Session

Session Chair: Sally A. Moody, George Washington University

SCGDB Lifetime Achievement Award

Reflections on the SCGDB and "Craniofacial Embryogenetics and Development"

Geoffrey H. Sperber

School of Dentistry, University of Alberta, Edmonton, Alberta, Canada

The Society of Craniofacial Genetics (SCG) was founded in 1975 under first President David Bixler (1929-2005) with the motto :"Educatio, Investigatio, Communicatio". The SCG held its inaugural meeting in 1975 in Baltimore, MD and initially published a Newsletter: "The Locus". For more details, please see the history page in this booklet.

The publication of the 3rd Edition of "*Craniofacial Embryogenetics and Development*" relates some of the most recent findings of the genetic basis of normal and defective development of the craniofacial complex. Melding the divergent disciplines of anatomy, embryology and syndromology provides an understanding of the developmental phenomena reflected in the disparities of development in craniofacial syndromes. Exquisitely timed confluent events are fundamental to normal development. Delayed or mistimed morphogenesis result in birth defects, exemplified in an exceptional case of a spontaneously healed cleft palate occurring post parturition.

David Bixler Excellence in Craniofacial Research Award

Cranial cartilage and bone formation in development and disease

Joan Richtsmeier

Department of Anthropology, Pennsylvania State University. University Park, PA.

Studying FGFR mutations associated with craniosynostosis has allowed our community to learn about bone development, and how separate osseous elements come together at sutures to form the bony skull in disease and in typically developing children. Early work focused on the closure of vault sutures and often analyzed this complex trait as an isolated phenomenon. Though of clinical significance, studies restricted to sutures ignores the importance of developmental context in the production of these traits. The use of mouse models for FGFR-related craniosynostosis conditions has enabled researchers to analyze prenatal events in the development of the skull and has revealed that traits other than sutures and tissues other than bone are affected in these craniosynostosis conditions. These observations provide novel avenues for research concerning the production of phenotypic variation in various cranial tissues and in the evolution of cranial phenotypes. We have turned our attention to the chondrocranium, the early cartilaginous skull that forms prior to the bony skull, providing protection for the early brain and sense organs. The chondrocranium of mouse models for FGFR-related craniosynostosis conditions are morphologically different from that of their typically developing littermates indicating that these mutations affect early cartilage formation. The connection of these cartilaginous anomalies to the development of bone – especially bone that forms intramembranously - is an open question.

Funding: NIH grants P01HD078233, R01DE027677

Marylou Buyse Excellence in Craniofacial Research Award

Developmental control of cell fate choice: insights from rare craniofacial disorders

Ryan R. Roberts[1], Lauren Bobzin[1], Diana Rigueur[1], Joanna E. Salva[1], Creighton T. Tuzon[1], Cynthia L. Neben[1], Amy E. Merrill[1*]

[1]Center for Craniofacial Molecular Biology, Ostrow School of Dentistry, University of Southern California, Los Angeles, CA, USA

Fibroblast growth factor 2 (FGFR2) acts as a key signaling node in the skeleton by regulating the binary choice of skeletal progenitors to either self-renew or differentiate. However, the mechanism by which FGFR2 controls these distinct cellular outcomes is not completely understood. Filling this gap in knowledge is necessary to uncover the pathophysiology of the 10 congenital skeletal disorders caused by *FGFR2* mutations. Our approach is to use disease-causing *FGFR2* mutations as a starting point for discovery and then build on this foundation using animal models. Our studies on Bent Bone Dysplasia syndrome (BBDS), a disorder characterized by craniosynostosis and bent long bones, have revealed a novel role of FGFR2 in the nucleolus, where it directly regulates ribosome biogenesis. We have shown that enhanced nucleolar FGFR2 activity in BBDS alters cell fate determination in skeletal progenitor cells by activating the nucleolar stress response. Our ongoing studies using the BBDS mouse model suggests that unregulated nucleolar FGFR2 triggers craniosynostosis by disrupting brain-bone interactions. In addition to BBDS, we are studying the mouse model for the *FGFR2* loss-of-function disorder LADD syndrome, which presents with jaw deformities. In these studies, we have found that FGFR2 is necessary for tendon-bone connectively in the jaw by establishing a gradient of tenochondral cell fate across the tendon-bone interface. Together, these studies have revealed novel and unexpected insights into the disease etiology of rare craniofacial disorders and also the normal function of FGFR2 in skeletal development.

Funding: NIH NIDCR (R01DE025222) and the March of Dimes (36-FY15-233 and FY12-166)

Molecular Regulation of Craniofacial Development

Session Chair: David Clouthier, University of Colorado Anschutz Medical Campus

New Genes Underpinning Evo-Devo Integration of Vertebrate Dentitions and Jaws

Julia C Boughner*, Cassy M Appelt, Nasim Rostampour, and Aunum Abid

Department of Anatomy, Physiology & Pharmacology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, S7N 5E5

Vertebrate teeth and jaws are incredibly diverse, but must still fit and function together for an animal to eat and survive. Quixotically, teeth and jaws have distinct evolutionary and developmental (evo-devo) origins, and yet their formation requires many of the same genes. For this reason alone, the evo-devo genetics that regulate tooth macroevolution and morphogenesis independently of the jaw skeleton have remained unclear. Now, by using a toothless p63-null mouse model with normal jaw formation, we are teasing out the genes integral for tooth but not jaw development. Our microarray, *in situ* hybridization and immunohistochemistry studies done in mouse at embryonic days 10-14 have discovered new genes, including Cbln1, Fermt1, Pltp and Prss8, expressed in dental epithelium. Next, we compared expression of these newly revealed genes in mouse molar organs to gene expression in the tooth organs of gar fish (*Lepisosteus oculatus*) at 14 days post-fertilization and frog (*Xenopus tropicalus*) at stage 57. Several genes expressed in mouse, including P63, Cbln1, Cldn23, Pltp and Prss8, were also expressed in gar and frog dental epithelium. The expression of the same genes in mouse, fish and frog teeth suggests that this p63-driven regulatory network is deeply conserved among living vertebrates, and has ancient evolutionary origins. This p63 gene regulatory network is also exciting because it may facilitate the macroevolution of dental phenotypes without necessitating change in the jaw skeleton.

Funding: Canada Foundation for Innovation grants and Natural Sciences & Engineering Research Council of Canada (NSERC) Discovery Grants to JCB; NSERC Undergraduate Student Research Award and Canada Graduate Scholarships to CMA, and; Saskatchewan Innovation and Opportunity, Canadian Institutes of Health Research-Techniques in Health Research Using Synchrotron Techniques, and Dept. of Anatomy Physiology and Pharmacology Scholarships to NR.

The primary cilia gene *Ttc21b* modulates craniofacial development as a crucial ciliopathy gene

Zakia Abdelhamed[1], David Paulding[1], Laura A. Runck[1], Sarah Salomone[1] and **Rolf W. Stottmann**[1,2*]

[1]Div.Human Genetics, Cincinnati Children's Hospital Med. Ctr, Cincinnati, OH USA
[2]Div. Devel. Biology, Cincinnati Children's Hospital Med. Ctr., Cincinnati, OH USA

The primary cilium is a critical signaling center and human patients with ciliopathies frequently suffer a number of congenital malformations, including orofacial phenotypes. Mouse *Ttc21b* mutants have multiple craniofacial phenotypes. In both humans and mice, ciliopathy phenotypes can be quite variable. We will share two approaches to identify the genetic basis of this phenotypic range. First, we have performed a Quantitative Trait Locus (QTL) analysis to explain some *Ttc21b* phenotypes which vary according to the background mouse strain. We have discovered a significant correlation between forebrain size and genetic background on distal chromosome 4. *Gpr63* is a candidate locus for this modifier and has been confirmed with an independent congenic backcross and direct modification of strain-specific *Gpr63* SNPs via CRISPR/CAS9 genome editing. Furthermore, *Ttc21b; Gpr63* double mutants have unique phenotypes consistent with novel genetic interactions. Secondly, in a ciliopathy patient cohort, *TTC21B* was previously found to be the most commonly mutated cilia gene (Davis et al., 2011). We have tested these associations from the human cohort with mouse models to examine how they affect craniofacial development. We have found that four genes identified in the human genetic studies (*Bbs7*, *Bbs10*, *Cc2d2a*, and *Mks1*) interact with *Ttc21b*. Doubly homozygous mutants have much more severe craniofacial phenotypes than either single mutant. Cells from *Ttc21b;Bbs7* and *Ttc21b;Bbs10* double mutants also show surprisingly drastic effects on ciliogenesis and signal transduction of crucial developmental signaling pathways. Most intriguingly, we find that heterozygosity for *Ttc21b* extends survival of *Bbs7* and *Bbs10* mutant mice. These findings have significant implications for models of oligogenic inheritance in ciliopathies and craniofacial genetics.

Funding: R01GM112744 and R35GM131875 (NIH/NIGMS) (R.W.S.).

Neural crest development is temporally regulated by miR-302

Rachel Keuls[1,2,3*] and Ronald Parchem[1,2,3]

[1] The Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX USA
[2]Department of Neuroscience, Baylor College of Medicine, Houston, TX USA
[3]The Stem Cells and Regenerative Medicine Center, Baylor College of Medicine, Houston, TX USA

Cranial neural crest cells are specified from ectoderm during gastrulation and possess a remarkable ability to generate diverse cell types. The mechanisms controlling the timing of neural crest formation are critical for proper development of craniofacial structures. Here, we identify two groups of miRNAs that counterpoise the transition from pluripotent ectoderm to multipotent migratory neural crest. Further, we find that miR-302 expression is maintained in migratory neural crest in mouse and is critical for regulating developmental timing of neural crest formation. Genetic rescue showed that miR-302 coordinates proper timing of neural crest formation by regulating expression of Sox9, a conserved neural crest specifier. Combined miRNA and mRNA profiling from gastrulation to the end of neurulation in mice revealed a set of genes co-targeted by multiple prevalent miRNA families. We used an unbiased screening to show that these co-targeted genes promote the formation of neural crest in chicken. Our findings reveal a post-transcriptional regulatory network that controls the balance between stemness and differentiation in neural crest cells.

Funding: CPRIT, V Foundation, B+ Foundation (R.P.)

Identification of novel candidate genes associated with SIX1 and Branchio-oto-renal syndrome.

Andre L. P. Tavares*, Karen M. Neilson, Himani D. Majumdar and Sally A. Moody

Department of Anatomy and Cell Biology, The George Washington University, School of Medicine and Health Sciences, Washington, D.C. USA

According to the CDC, the prevalence of hearing loss is 1.4 per 1000 babies screened in 2009 in the USA. Branchio-oto-renal (BOR) syndrome is an autosomal dominant disorder characterized by hearing loss, branchial fistulas/cysts, and renal anomalies in about a third of patients. Mutations in the *SIX* gene and the SIX co-factor *EYA1* gene are present in half of BOR patients. We are using *Xenopus laevis* and mouse as models to discover novel genes involved in this syndrome. Based on a screen in fly of proteins shown to interact with *So* (*Drosophila* Six1 homologue), *sobp*, *zmym2* and *zmym4* were identified as potential Six1 co-factors. Expression data show that these genes are expressed in patterns that overlap with *six1* during craniofacial development. Experiments using luciferase assays to test if these factors are able to modify Six1 function show that Sobp or Zmym2 repress Six1+Eya1 transcriptional activity, whereas Zmym4 enhances it, indicating these factors functionally interact with Six1. Knockdown experiments using morpholino antisense oligonucleotides and CRISPR/Cas9 followed by ISH and qPCR analyses show that Sobp is required for neural border zone development (*pax3*, *msx1*, *tfap2*) and later for neural crest (*foxd3*) and placode (*six1*) formation. These changes correlate with severe craniofacial defects in the tadpole. Knockdown of Zmym2 or Zmym4 suggests these factors are required for proper formation of the neuroectoderm/ectoderm (*krt12.4*) domains. These results indicate that Sobp, Zmym2 and Zmym4 are required for proper craniofacial development, and that they modify Six1 function, suggesting they may be potential candidate genes for BOR.

Funding: R01DE026434 (NIH/NIDCR) (S.A.M.), R03DE028964 (NIH/NIDCR) (A.L.P.T.), GWU SMHS

Evolution of vertebrate gill covers through shifts in an ancient gnathostome Pou3f3 enhancer

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The evolution of jaws was accompanied by a major change in respiratory strategy. Whereas the gill cavities of extant jawless vertebrates like lamprey open directly into the environment, jawed vertebrates evolved endoskeletal appendages that protect and promote unidirectional flow of oxygenated water over the gills. In bony fish, a single appendage, or operculum, covers a large common gill cavity. In contrast, most cartilaginous fish have separate covers for each gill. We find that these divergent gill cover patterns correlate with the expression of Pou3f3 orthologs in the pharyngeal arches. We identify a potent Pou3f3 arch enhancer that is deeply conserved across nearly all jawed vertebrates but undetectable in lamprey and non-vertebrate chordates. Remarkably, small sequence differences within this enhancer explain the restricted bony fish versus pan-arch cartilaginous fish expression patterns. Using zebrafish *pou3f3a/b* mutants, we find that Pou3f3 is essential for sustained posterior-directed outgrowth and endoskeletal development of the gill cover. Reciprocally, forced expression of Pou3f3b in the posterior arches generates ectopic gill-associated skeletal elements. Emergence and modification of this ancient enhancer driving Pou3f3 arch expression may thus have helped transform the pharyngeal bauplan during early gnathostome evolution.

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Cell Biology of Craniofacial Development

Session Chair: Lisa Taneyhill, University of Maryland College Park

Using frog faces to dissect mechanisms underlying human orofacial defects

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Xenopus has several qualities that make it advantageous for craniofacial research: **1)** The free-living embryo has an easily accessible face, lacking significant head flexure that is present in other models. For this reason screening embryos for craniofacial defects is relatively easy. Such screens have allowed us to determine that embryos deficient in retinoic acid over a specific window of development form orofacial clefts that are similar to median oral clefts in humans. Using *Xenopus*, we are developing an in-depth understanding of the molecular and cellular mechanisms underlying such orofacial malformations; **2)** Current tools used in *Xenopus* such as morpholinos and mosiac Crispr/Cas9-mediated mutagenesis can be used to alter gene dosage, rather than completely knocking-out the gene. These tools have allowed us to model CNVs associated with craniofacial birth defects. Such experiments are not only validating a role for a particular gene in the disease, but are also increasing our understanding of how the gene product regulates development of the orofacial region; **3)** The *Xenopus* embryonic skin has similar barriers as the mammalian placenta and lungs making it a relatively efficient tool to study inhaled environmental toxins. Therefore, we have been using *Xenopus* to determine if and how components of e-cigarettes could affect craniofacial development. In summary, *Xenopus* is an effective tool to begin to uncover both genetic and environmental factors that can contribute to human orofacial defects.

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Ronin (Thap11) regulates neural crest and craniofacial development via the vitamin B_{12} pathway

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Craniofacial malformations are a significant medical issue affecting 1 in 3 babies born with a birth defect. The neural crest (NC) gives rise to the majority of the craniofacial structures and thus defects during its development underlie the majority of craniofacial malformations. The NC is a transient, multipotent, migratory cell population that besides giving rise to the craniofacial skeleton, it also contributes to a diverse array of tissues including neurons and melanocytes. Although a gene regulatory network governing the various stages of NC development including specification, epithelial-to-mesenchymal transition, migration and differentiation has been revealed, little is known about its multipotency in mammals. Here we report a novel role for the transcriptional regulator *Ronin* in NC and craniofacial development in the mouse. Strikingly, conditional knock-out (CKO) of *Ronin* in the NC, results in agenesis of the facial skeleton, whereas other NC derivatives appear normal, suggesting a specific role of *Ronin* during osteochondrogenesis. Specification, EMT, and migration of the NC seem normal in the CKO, but cell death in the craniofacial primordia suggests a failure of NC differentiation into cartilage and bone. Moreover, *Ronin* has been implicated in the rare vitamin B_{12}-deficiency disorder cblX. Consistent with our findings, these patients exhibit craniofacial abnormalities. This is the first study to reveal a role of *Ronin* and vitamin B_{12} in the NC and craniofacial development. Thus, continued investigation using the *Ronin* CKO will be crucial in revealing the pathophysiology of the vitamin B_{12}-deficiency disorder as well as better understanding the multipotency of the NC.

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Srsf3-mediated alternative RNA splicing downstream of PDGFRα signaling in the palatal mesenchyme

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Craniofacial development is a critical morphological event during embryogenesis, defects in which result in highly prevalent human birth defects. In humans and mice this process relies on signaling through the platelet-derived growth factor receptor alpha (PDGFRα). Mutations in human *PDGFRA* are associated with cleft lip/palate and mouse models with mutations in this gene similarly display facial clefting phenotypes. PI3K is the main downstream effector of PDGFRα signaling during mouse development. We previously performed a mass spectrometry-based phosphoproteomic screen to identify targets of PI3K/Akt-mediated PDGFRα signaling in primary mouse embryonic palatal mesenchyme cells (MEPMs), revealing an enrichment for RNA-binding proteins. We hypothesize that one of these Akt phosphorylation targets, Srsf3, mediates tissue-specific alternative RNA splicing downstream of PDGFRα signaling in the palatal mesenchyme. We have biochemically confirmed the PI3K/Akt-mediated phosphorylation of Srsf3 upon PDGF-AA ligand treatment of MEPMs and further demonstrated that this phosphorylation drives Srsf3 translocation into the nucleus. We revealed that expression of Srsf3 is enriched in the maxillary processes and palatal shelves of mid-gestation mouse embryos. Moreover, RNA-sequencing analysis of palatal shelf mesenchyme derived from wild-type versus autophosphorylation mutant knock-in embryos in which PDGFRα is unable to bind PI3K identified differentially alternatively-spliced transcripts containing Srsf3 binding sites that are associated with craniofacial defects. Finally, we showed that ablation of *Srsf3* in the neural crest lineage results in embryos with midline facial clefting, facial bone hypoplasia and exencephaly. Taken together, our results point to a novel role for the PDGFRα-PI3K/Akt-Srsf3 signaling axis in regulating RNA processing during craniofacial development.

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Paracrine activation of neural crest EMT genes by placodal MMP28

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Matrix Metalloproteinase are a family of proteases most known for their matrix remodeling activity in several cancers and chronic inflammatory diseases. However, MMPs are also found in intracellular compartments notably the nucleus of several cell types. MMP28 is the last discovered member of the MMP family. It is involved in wound repair, central nervous system development, and immune system maturation. In addition, MMP28 upregulation has been linked to several cancers. MMP28 activity can induce epithelial-mesenchymal-transition (EMT) and migration in human alveolar epithelium and is found in the cell nuclei. Very little is known about the nuclear activity of MMPs or how MMPs are shuttle within the cells. In *Xenopus laevis* embryos, MMP28 is expressed in the pre-placodal region (PPR) and medial neural crest (NC) at the end of gastrulation (st. 12.5/13) and persists in the epibranchial placodes between the NC streams. Interestingly, we have shown that MMP28 secreted by the placodes is found in the nuclei of neighboring NC cells. MMP28 knockdown caused a strong reduction of expression of several transcription factors regulating NC cells EMT. Later these cells failed to properly migrate both *in vitro* and *in vivo*. Furthermore, we demonstrated that MMP28 function is dependent on its nuclear catalytic activity. All together these results suggest a paracrine activation of EMT transcription factors by placodal MMP28 to regulate NC development.

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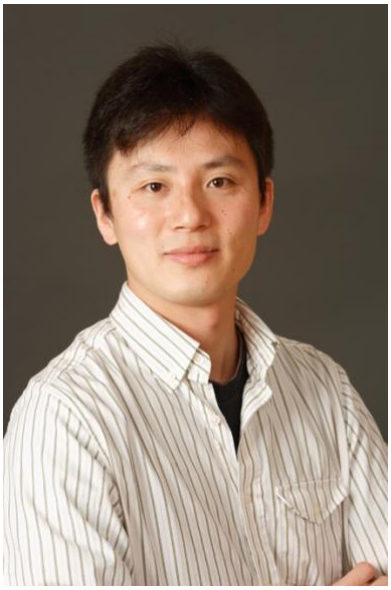

Cholesterol-dependent primary cilium formation is crucial for osteogenesis

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Genetic mutations related to cholesterol metabolism or high-cholesterol maternal diets are considerable risk factors of craniofacial deformities. However, it is largely unknown the role of intracellular cholesterol metabolism in craniofacial development. Here we show that an altered cholesterol metabolic status results in abnormal osteogenesis through dysregulation of primary cilia, which are responsible for sensing extracellular cues, during intramembranous ossification. We found that cholesterol metabolic aberrations, induced through disruption of either *Dhcr7* (which encodes an enzyme involved in cholesterol synthesis) or *Insig1* and *Insig2* (which provide a negative feedback mechanism for cholesterol biosynthesis), result in altered born formation. We found that the formation of primary cilia were decreased and increased in number and length in *Dhcr7* and *Insig1/2* mutant osteoblasts, respectively. As a consequence, WNT/β-catenin and hedgehog signaling activities were altered through dysregulated primary cilium formation. Importantly, the normalization of altered cholesterol metabolism rescued the abnormalities in both ciliogenesis and osteogenesis in these mutant mice. Thus, our results indicate that proper intracellular cholesterol status is crucial for skull formation and homeostasis through primary cilium formation.

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Translational Craniofacial Biology

Session Chair: Timothy Cox, University of Missouri-Kansas City

Insights from large-scale discovery of mammalian essential genes

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While the pace of human disease gene discovery is accelerating, ascribing causality to a given gene variant is often challenging and thus many cases remain unsolved. The goal of the International Mouse Phenotyping Consortium (IMPC) is to help bridge this gap through the systematic functional annotation of the mouse genome, including large number of genes for which there is little or no experimental data. To date, over 8,500 individual gene knockout mice have been produced, of which we have found that approximately 1/3 are essential for life. Using a high-throughput embryonic phenotyping pipeline, we have uncovered numerous novel developmental phenotypes, including craniofacial dysmorphology. Our large-scale dataset has further refined of a core mammalian “essentialome” which is highly enriched in human disease genes, thus providing a novel dataset that facilitates prioritization of mutations identified in clinical sequencing efforts. While CRISPR/Cas9 genome editing has accelerated pipelines for mouse model creation and phenotyping, the process is relatively slow and costly. To address this issue and taking advantage of the IMPC embryo phenotyping pipeline, we have developed a rapid, scalable platform to directly evaluate the causality of novel genes/variants for developmental disorders. In proof-of-principle studies, we demonstrate the feasibility of direct phenotypic analysis of F0 (“founder”) mouse embryos generated by CRISPR/Cas9, and show that we can efficiently generate and identify expected morphological phenotypes in mosaic edited embryos. Thus, our platform has the potential to improve the speed and utility of the mouse as a tool to validate and model novel variants for human disease.

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A novel role for the mediator complex protein Med23, in craniofacial development and in the pathogenesis of Pierre-Robin Sequence

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Pierre Robin Sequence is a congenital craniofacial disorder characterized by micrognathia, glossoptosis and cleft palate, the combination of which leads to difficulty breathing and feeding early in life. With an incidence of 1 in 5,500 live human births in the United States, our knowledge of the genetic causes for the disorder is currently limited to mutations in a highly conserved non-coding region of Chromosome 17 in humans. This region contains enhancers for *SOX9*, a transcription factor that regulates neural crest cell and cartilage development and *KCNJ2,* a member of Potassium Voltage-Gated Channel subfamily of proteins. However, most cases of Pierre Robin Sequence malformation have an unknown genetic diagnosis, making it imperative to identify other genes and biological processes that contribute to the etiology and pathogenesis of this disorder. We have identified a new gene *Med23,* the loss of which in neural crest cells (*Med23*$^{fx/fx}$*;Wnt1-Cre)* results in hypoplastic upper and lower jaw and cleft palate, mimicking Pierre Robin Sequence. *In-situ* hybridization and immunostaining revealed that both *Sox9* mRNA and protein levels were upregulated in *Med23*$^{fx/fx}$*;Wnt1-Cre* embryos compared to controls. Altogether, this data supports a cell-autonomous requirement for Med23 in neural crest cell and craniofacial development, potentially linking Med23 to the etiology and pathogenesis of Pierre Robin Sequence.

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A novel 3 MB deletion on 6p24 excluding TFAP2A results in a mild phenotype in a multiplex family with orofacial clefting

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Nonsyndromic orofacial clefting (OFC) has a complex etiology but multiplex families are likely to be enriched for high effect variants that reflect novel genetic factors or causal mechanisms. We identified a novel 3 Mb deletion on 6p24 in three affected members of the same family from Colombia, with a reported dominant inheritance pattern. All affected family members with DNA (4 of 5 individuals, including one not typed in the GWAS) carried the deletion. Exome sequencing of this family found no segregating single nucleotide variants in OFC candidate genes, supporting the pathogenicity of this deletion. The 3Mb deleted region included 12 genes and a gene desert spanning approximately 840 kb, however none were strong OFC candidates. The 3' breakpoint of the deletion is located in the middle of a conserved 2.5Mb topologically associated domain (TAD) containing *TFAP2A*. In addition to removing the 5' boundary of the TAD, the deletion includes one enhancer active in human cranial neural crest cells and human fetal craniofacial tissue and a second with activity in neural crest in mouse embryos. *TFAP2A* mutations and deletions cause Branchiooculofacial Syndrome (BOFS), a dominant syndrome that includes OFC as a primary phenotypic feature. Except for OFC and characteristic facial features (broad nasal root and slight hypertelorism), the affected members of this family lack additional features of BOFS. Cumulatively, these data suggest that deletion of distal *TFAP2A* regulatory elements result in a craniofacial phenotype consistent with mild features of BOFS and that these elements are excellent candidates for nonsyndromic OFC etiology.

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Role of MMP2 in early craniofacial development in zebrafish

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Defects in early craniofacial development can lead to non-syndromic cleft lip and palate (NSCLP), which affects 4,000 newborns in the US annually. Candidate gene analysis and whole exome sequencing of pedigrees of affected and unaffected individuals reveal both an environmental and genetic contribution to NSCLP. Previous work in our lab using zebrafish identified *crispld2* as a biologically relevant NSCLP gene. Perturbation of *crispld2* resulted in altered migration and proliferation of neural crest cells (NCCs). To further elucidate the role of *crispld2*, we used RNA-seq to identify genes that were differentially expressed between wild type and *crispld2* morphants. One of these genes is matrix metallopeptidase 2 (*mmp2*). We assessed association of variants in *MMP2* with human NSCLP and found significant association between NSCLP and *MMP2*/rs243836 (p=0.002) in our Hispanic families. Morpholino knockdown of *mmp2* in zebrafish results in embryos with smaller head, eyes and body axis and abnormal mandibular arch skeleton at low concentrations and severe cardiac edema, curved body axis and death at higher concentrations. The viscerocranium is derived from cranial NCCs that populate the seven pharyngeal arches of the zebrafish embryo. With evidence of *crispld2* in affecting NCC migration, these preliminary experiments point to a CRISPLD2-mediated role of MMP2 in the development of craniofacial skeleton. Our study provides a model to test putative genes associated with NSCLP *in vivo*, explore their molecular mechanism in early orofacial development and identify new targets that can be tested in our human NSCLP families.

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Integrated analysis of RIC1 mutations and phenome in a biobank identifies novel craniofacial syndrome and collagen trafficking mechanisms

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Discovery of genotype-phenotype relationships remains a major challenge in clinical medicine and basic biology. Here, we examined three sources of phenotypic data to uncover a novel craniofacial syndrome and disease pathomechanism. Using a zebrafish phenotype-driven forward genetic screen we identified the *ric1* gene to be essential for normal craniofacial biology. We show that activation of Rab6a GTPase by the Ric1-Rgp1 GEF complex is required for procollagen secretion through the Trans-Golgi-Network (TGN) *en route* to extracellular matrix. Using a gene-based phenome-wide association study (PheWAS) in the EHR-linked BioVU biobank, we show reduced genetically determined expression of *RIC1* is associated with common disease phenome that includes skeletal and dental conditions. Individuals homozygous-by-descent for a rare variant in *RIC1* underwent a guided re-evaluation of their clinical features, and were discovered to have many signs of the BioVU associated phenome. We have termed this novel Mendelian craniofacial syndrome CATIFA (Cleft lip, cAtaract, Tooth abnormality, Intellectual disability, Facial dysmorphism, ADHD). Zebrafish cells and CATIFA patient skin fibroblasts accumulate intracellular procollagen in TGN, and the human *RIC1* variant fails to rescue the procollagen secretory defects in zebrafish mutants, unlike wild type *RIC1,* establishing pathogenicity of the variant and evolutionary conservation of this procollagen secretory pathway. Taken together, our data indicate that RIC1-RGP1 complex is essential for collagen secretion and normal craniofacial development.

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Signaling during Craniofacial Development

Session Chair: Jean-Pierre Saint-Jeannet, New York University

Developmental genetic regulation of the mammalian inner ear

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The mammalian inner, middle and outer ears have different embryonic origins, yet the development of each component of the auditory apparatus must be precisely synchronized in space and time. We have identified a Forkhead transcription factor, Foxi3, that is expressed at very early stages in the embryonic head. Foxi3 mouse mutants made in our lab lack all components of the inner, middle and external ears. Our work suggests that one of the first steps in ear induction– the formation of the otic placode – does not occur in Foxi3 mutants. Our analysis of Foxi3 mutant mice suggest that Foxi3 may act *positively* as a pioneer factor to prepare ear genes for transcription in response to inducing signals. However, mutation of Foxi3 also causes the up-regulation of Hox genes that impart positional information to the CNS and neural crest, but which are normally repressed in placodes. This suggests that in addition to its *positive* pioneer factor activity, Foxi3 also acts *negatively* in the placodal region to repress genes that normally pattern the CNS and neural crest.

We have shown that Foxi3 regulates at least one factor, FGF8, which is critical for the survival of the neural crest cells that contribute to the middle and outer ears. We recently identified a series of human patients with point mutations or deletions in Foxi3, some of whom display phenotypes such as microtia and conductive hearing loss. Interestingly, one of these affected families has a variant immediately adjacent to the Foxi3 nuclear localization signal recently characterized by our lab, suggesting that Foxi3 variants may underlie previously undiagnosed outer and middle ear defects.

TBC1d24-ephrinB2 interaction regulates contact inhibition of locomotion in neural crest cell migration

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Missense mutations are an underlying cause of DOORS (Deafness, Onychodystrophy, Osteodystrophy, mental Retardation, Seizures) syndrome in patients. The syndrome manifests as an intellectual disability, seizures, deafness, short distal phalanges, small or absent nails, occasional dysmorphic facial and cranial features. However, the function of TBC1d24 in cellular signaling during vertebrate embryonic development is unclear. We provide evidence that TBC1d24, a putative Rab35-GTPase activating protein (Rab35 GAP), is expressed in cranial neural crest (CNC) cells and complexes with ephrinB2 via the major Wnt signaling scaffold Dishevelled (Dsh) and mediates a signal affecting contact inhibition of locomotion (CIL) in CNC cells. Moreover, we found that, in migrating CNC, the interaction between ephrinB2 and TBC1d24 negatively regulates E-cadherin recycling in these cells via Rab35. Upon engagement of the cognate Eph receptor, ephrinB2 is tyrosine phosphorylated, which disrupts the ephrinB2/Dsh/ TBC1d24 complex. The dissolution of this complex leads to increasing E-cadherin levels at the plasma membrane, resulting in loss of CIL and disrupted CNC migration. Our results indicate that TBC1d24 is a critical player in ephrinB2 control of CNC cell migration via CIL.

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Hippo pathway interacts with Wnt pathway to control the neural crest cells

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The neural crest (NC) is a transient and migratory multipotent cell population that can differentiate into different derivatives. Hippo pathway is a fundamental pathway, yet its role in NC is still poorly understood. Here, we found the Hippo effectors *Yap* and *Taz* are required for NC cells proliferation, migration and differentiation, based on a comprehensive analysis using *in vivo* NC conditional knock-out mouse models and *in vitro* NC cells. *Yap* and *Taz* deficiency caused vascular and cranial bone defects in mice and failure of NC cells to migrate and differentiate. Wnt-β-catenin pathway, another fundamental pathway, has been shown to play a key role in regulating NC cells. Our motif analysis of ATAC-seq (assay for transposase-accessible chromatin with high-throughput sequencing) data in mouse craniofacial tissues and human NC cells indicated that genome-wide, 38.9% genes have enriched Tead (Yap/Taz cofactor) motifs are also enriched in Tcf/Lef (β-catenin cofactors) motifs, suggesting Hippo interacts with Wnt to coordinately regulate gene expression in NC cells. Our co-IP data uncovered a direct interaction between Yap/Taz and β-catenin in NC cells. Furthermore, our RNA-seq data in mouse craniofacial tissues and Reverse Phase Protein Array (RPPA) data in mouse NC cells indicated that Wnt target genes important for cell migration and fate determination such as *Twist1* and *Sox9* were also regulated by Yap/Taz. Yap/Taz-Tead and Tcf/Lef-β-catenin complexes potentially coordinately and directly regulate the expression of these genes. Together, our findings uncover novel roles of hippo-Yap pathway in regulating NC cells, which partially functions through interaction with Wnt-β-catenin pathway.

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Functional effects of *WNT10A* rare variants identified in individuals with isolated tooth agenesis

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Tooth agenesis (TA) is a common craniofacial anomaly affecting ~200 million humans worldwide that results from failure of permanent tooth development. TA presents considerable phenotypic and genotypic heterogeneity, and animal model systems do not always recapitulate the phenotype in humans. *WNT10A* has been suggested as a major gene for TA, and yet the biological effects of identified variants and their potential phenotypic implications in TA remain unknown. Moreover, phenotypes in animal models of *Wnt10a* knockdown/knockout are contradictory. In this study, we evaluated the effects of 13 rare *WNT10A* variants previously associated with TA using computational modeling and functional validation in a patient-based *in-dish* model system. Plasmid constructs for wild-type and mutant *WNT10A* were transfected into SHED (stem cells of exfoliated deciduous teeth) cells for evaluation of *WNT10A* gene/protein expression, WNT signaling, and effects on expression of additional tooth development genes. Ten WNT10A variants showed significantly lower or no luciferase activity in comparison to wild type suggesting that the truncated protein failed to activate or perturbed WNT signaling.WNT10A mutant cells also presented decreased WNT10A protein expression and reduced ability to bind to FZD, in addition to altered expression of *PAX9, MSX1, AXIN2,* and *RUNX2.* RNA-seq of p.T357I-mutant cells in comparison to wild type identified 38 differentially expressed genes belonging to pathways related to cell pattern specification/regionalization and adaptive immune response. Our results suggest that distinct *WNT10A* variants may present different biological effects contributing to impaired gene/function which may in turn contribute to milder or more severe TA phenotypes.

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Poster Abstracts

Poster #1

Mapping inhibitory cascade related genetic effects on murine molars

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The inhibitory cascade (IC) model proposes that the evolution of relative molar sizes in eutherian mammals is largely governed by the antagonistic effects of activating and inhibiting influences on molar growth. We demonstrate that this model of developmental influences on the relative sizes of molar teeth translates into precise predictions about the quantitative effects of genes on molar sizes. Using high-resolution quantitative trait locus mapping in an advanced intercross experimental population of mice, we tested whether the IC model explains genetic effects on individual differences in tooth morphology on a locus by locus basis. We detected 63 loci with robustly estimable genetic effects on one or more of the molars. A sizeable minority of loci we detected (24 from 63) have effects on teeth that make them candidates for being involved in an IC like process. That is, they had robust effects on the first molar with appropriately proportioned effects on the other teeth. This lends support to the contention that IC related process shape variation in mammalian molars. Other mechanisms, however, contribute substantially to within-population variation as demonstrated by the fact that 39 of 63 loci have genetic effects that deviate from IC model expectations in some way. These other sources of variation give teeth considerable latitude to vary in ways at odds with the expectations of the IC model.

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Poster #2

The role of *FZD6* and related genes in craniofacial morphogenesis

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Craniofacial birth defects are the most common structural abnormalities at birth. They include nonsyndromic cleft lip and palate (NSCLP), which has a prevalence of 1 in 700 and affects approximately 4000 newborns in the United States annually. Although many genes are implicated in NSCLP etiology, only a portion of the heritability has been explained. We have previously identified *FZD6*, a receptor in the Wnt pathway, as an NSCLP gene in a large multigenerational family and further shown that perturbation of *fzd6* causes cartilage and bone abnormalities in zebrafish. Additionally, others have shown that loss of co-receptor *lrp6* causes bilateral cleft lip in mice, while knockdown and mutant forms of *lrp5* cause abnormal craniofacial cartilage phenotypes in zebrafish. Furthermore, the facial region completely fails to form in mice deficient for *dkk1,* a Wnt antagonist. These genes collectively act at the receptor level of β-catenin mediated Wnt signaling, which plays an important role in craniofacial morphology.

The goal of this study was to evaluate the effects of gene dosage perturbations for *fzd6* and related genes in zebrafish using craniofacial morphometry. First, we evaluated β-catenin activation during craniofacial development utilizing a stable zebrafish reporter line. Reporter expression was analyzed from 1-7 days post fertilization (dpf) and was highly active in the oral cavity and other facial regions between 3-5 dpf. As development progressed, β-catenin expression became restricted to cells around the peri-oral region. Upon *fzd6* knockdown, morphometric analysis revealed that head width ($p=0.0002$), olfactory distance ($p=0.0002$), mouth perimeter (<0.0001) and mouth width ($p=0.0005$) were significantly reduced while mouth height was increased (0.006) in morphant embryos compared to controls. *Lrp5*, *lrp6* and *dkk1b* knockdown led to similar changes. The results of this study provide important information about the effects of *FZD6* and related β-catenin/Wnt pathway genes in craniofacial development and morphogenesis.

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Poster #3

The contribution of de novo mutations identified by WGS of 643 trios with orofacial clefts

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Orofacial clefts (OFCs) are the most common craniofacial malformation in humans with a substantial genetic component to etiology. About 15% of all OFC cases have a family history of OFC, supporting a contribution of inherited genetic variants. However, the remaining cases occur sporadically, suggesting a role for *de novo* mutations (DNMs). A handful of DNMs have been reported, but their overall contribution to OFCs has not been thoroughly assessed in a large sample and on a genome-wide scale. Therefore, we performed whole-genome sequencing on 643 European or Latino case-parent trios with nonsyndromic OFCs and developed a stringent filtering pipeline to generate a list of high confidence DNMs. Of the 58,735 DNMs identified genome-wide, 757 were exonic (~1.2 exonic DNMs per trio). Genes carrying DNMs in OFCs were significantly enriched for terms related to relevant biological processes (embryo development, embryo morphogenesis), mouse phenotypes (abnormal embryo morphology), and human disease (cleft). Several genes showed multiple DNMs, including known OFC risk genes (*IRF6*, N=3; *CTNND1*, N=2) and novel candidates (*ZFHX4*, N=2, *RAP1GAP*, N=2). Notably, predicted loss-of-function DNMs were found in multiple genes mutated in Mendelian OFC syndromes, but individuals in this cohort carrying these mutations lacked additional features required for clinical diagnosis of a syndrome (e.g., *CHD7*, *COL2A1*, *CTNND1*, *TFAP2A*, and *RPL5*). Additionally, we found missense DNMs in OFC candidate genes previously identified through genome-wide association studies or animal models: *FAF1*, *THADA, and SHROOM3.* Cumulatively, this study provides the strongest evidence to date for a role for DNMs in the etiology of OFC.

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Poster #4

Finding MEMO—tissue specific functions of MEMO1 during craniofacial mineralization

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Many genetic diseases impact the development and function of mineralized tissues in the cranium (e.g. bones and teeth). Despite this critical importance, our knowledge of this process is superficial, particularly compared to the bones of the trunk. Part of this disconnect stems from the differing embryonic origins of mineralized tissues of the craniodental complex, including neural crest cells (NCCs), presomitic mesoderm, and oral ectoderm, in contrast to the somatic mesoderm derived trunk skeleton. Understanding the unique gene regulatory networks and cellular and molecular mechanisms driving mineralization of craniodental tissues is an essential step in explaining, preventing, and treating associated genetic disorders, and harnessing this information for tissue regeneration. Here, we present evidence that the protein, encoded by the gene *Memo1*, has a multifaceted role in craniofacial tissue mineralization. Using conditional-mouse genetics, we identify that MEMO1 operates within both a neural crest cell as well as an ectoderm-specific program of bone and enamel mineralization, respectively. Through a combination of bulk and single cell RNA-sequencing, immunohistochemistry, SEM, µCT, and basic histological examination we are dissecting the molecular mechanisms underlying MEMO1 function during these processes. Interestingly, despite the unique embryological origins of both craniofacial bone and enamel, we find that similar facets of MEMO1 function appear to be deployed in both tissues. We are carrying out additional studies to further define the broader network in which MEMO1 functions and how these processes might be exploited in the context of tissue regeneration.

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Poster #5

Characterizing the role of Pdgfra in mouse chondrocranium development

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The chondrocranium is composed of multiple cartilages of the craniofacial region. Although it is well known that chondrocranium is critical for normal development, the mechanisms of its formation remain largely unknown. Our data show that Platelet derived growth factor receptor alpha (*Pdgfra*) null mutant mice exhibit severe phenotype in craniofacial cartilages, indicating that *Pdgfra* plays a crucial role in its development. However, tissue-specific manipulation of Pdgfra activity in the chondrocytes only causes mild phenotype. Interestingly, we found that *Pdgfra* null mutant exhibits modest change in cell proliferation and survival, but significant decrease in expression of *Sox9* and *Col2a1*, suggesting a role for *Pdgfra* in early progenitor formation. To test this hypothesis, we have isolated embryonic mesenchymal stem cells (eMSCs) from E13.5 embryonic heads, and verified the capacity of these cells differentiating into multiple cell types. Activating Pdgfra signaling in these cells significantly enhances transcription of *Sox9* and *Col2a1*, showing that Pdgfra promotes multipotent eMSCs differentiating towards chondrocytes. In $Sox9_{+}$ chondrocyte progenitors, we identified that Pdgfra activation decreases expression level of *Wnt9a* and other Wnt signaling target genes, which are known in directing stem cell fate and chondrocranium development. We have further confirmed that Pdgfra regulates *Wnt9a* transcription in developing craniofacial cartilages *in vivo*. Moreover, our data demonstrated that activating Wnt signaling partially rescue ectopic cartilage formation caused by excessive Pdgfra activity in embryos. In summary, our data show that Pdgfra plays a critical role in multiple stages of craniofacial chondrocyte development.

Poster #6

Mutation spectrum in 147 Chinese patients with craniosynostosis: findings of a custom sequencing panel study

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Craniosynostosis (CS), one or more of the cranial sutures in an infant skull prematurely fuses. Most genetic studies on CS were carried out in Caucasian populations, while only a few case reports were performed in East Asian populations. In our study, we established a Chinese craniosynostosis patient cohort without prior molecular diagnosis. We aimed to assess the impact of a targeted gene sequencing for the genetic diagnosis, and to describe the characteristics of mutation spectrum in Chinese craniosynostosis patients. We firstly designed a panel to sequence exonic regions of 17 genes based on a comprehensive literature review. We then performed panel sequencing and bioinformatic analysis in 147 probands. The confirmation and inheritance of suspected variants were validated in probands and their available parents by Sanger sequencing. Variants were identified according to the ACMG standards, based on silico prediction and Sanger validation. A total of 80 cases were identified with pathogenic or likely pathogenic variants. In total, we identified 43 different variants in 9 genes (*EFNB1, ERF, FGFR1, FGFR2, FGFR3, POR, TCF12, TGFBR2, TWIST1*). We further showed that the distribution of variants has no significant differences between Chinese and Caucasian patients of Apert, Crouzon and Pfeiffer syndromes. However, in Crouzon patients, the mutation frequency at the C342 and Y340 residues were significantly different between the two populations. In addition, eleven novel variants were identified, which had never been reported in Caucasian patients. In summary, our custom sequencing panel can provide reasonably high-yield diagnosis in Chinese craniosynostosis patients. The differences and novel findings of our study suggested great potential to carry out systematic genetic studies in Chinese craniosynostosis patients.

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Poster #7

Mapping the relationship between proliferation and morphology in the mouse face

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There is a long-standing prediction that small changes in proliferation and apoptosis during the time frame of facial morphogenesis act to shape the face. Here, we set out to test that prediction by quantifying proliferation and apoptosis in 3D and relating it to the growth of the face. We use whole mount staining for proliferation and apoptosis markers, whole tissue clearing methods, lightsheet microscopy and atlas and machine learning based quantification methods to identify individual proliferating or apoptotic nuclei within a 3D tissue structure at a set time point. We also employee geometric morphometric analysis of the same tissue structure to quantify overall morphology. By collecting data at various time points across facial development (E9.5-E11.5) and quantifying the age of each embryo, we are able to relate cell biological level growth to tissue level growth and morphological change and relate these two parameters in a way not performed previously.

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Poster #8

Precocious chondrocyte differentiation disrupts skeletal growth in Kabuki syndrome mice

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Kabuki syndrome 1 (KS1) is a Mendelian disorder of the epigenetic machinery caused by mutations in the gene encoding KMT2D, which methylates lysine 4 on histone H3 (H3K4). KS1 is characterized by intellectual disability, postnatal growth retardation, and distinct craniofacial dysmorphisms. A mouse model (*Kmt2d*$^{+/\beta Geo}$) exhibits features of the human disorder and has provided insight into other phenotypes; however, the mechanistic basis of skeletal abnormalities and growth retardation remains elusive. Using high-resolution micro-computed tomography we show that *Kmt2d*$^{+/\beta Geo}$ mice have shortened long bones and ventral bowing of skulls. *In vivo* expansion of growth plates within both the skull and long bones suggests an abnormal rate of cell proliferation during endochondral ossification as a common disease mechanism. Stable chondrocyte cell lines harboring inactivating mutations in *Kmt2d* exhibit increased proliferation and differentiation, which further supports this mechanism. A known inducer of chondrogenesis, SOX9, and its targets show markedly increased expression in *Kmt2d*$^{-/-}$ chondrocytes. By transcriptome profiling, we identify *Shox2* as a putative KMT2D target. We propose that decreased KMT2D-mediated H3K4me3 at *Shox2* releases *Sox9* inhibition and thereby leads to enhanced chondrogenesis, providing a novel and plausible explanation for precocious chondrocyte differentiation. Our findings not only provide insight into the pathogenesis of growth retardation in KS1, but also suggest novel therapeutic targets to rescue growth retardation in KS1 and related disorders.

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Poster #9

Chondrocyte polarity requires protein-protein interactions between *Prickle1* and *Dishevelled 2* and *3* during endochondral ossification

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The organized, polarized maturation of chondrocytes in the growth plate is necessary for the growth of the limb and cranial base. Decreased growth plate expansion is linked to shorter stature and midfacial hypoplasia, but the underlying molecular pathologies are not well described. We observed that *Prickle1*$^{Beetlejuice/Beetlejuice}$ mutant mice develop midfacial hypoplasia and shorter limbs. The *Prickle1*^{Bj} mouse line has a missense mutation (c.G482T:p.C161F) that disrupts a cysteine knot in the LIM1 domain resulting in a hypomorphic protein. *Prickle1* is a core component of the Wnt/Planar cell polarity (Wnt/PCP) pathway. We found that the *Prickle1*$^{Bj/Bj}$ chondrocytes have randomized localization of Dishevelled2 (Dvl2) and Dvl3 proteins. The abnormal protein localization resulted in randomization of chondrocyte polarity and the failure to organize to columns in the *Prickle1*$^{Bj/Bj}$ growth plate. Importantly, we observed *in vivo* protein-protein interactions between Prickle1 and either Dvl2 or Dvl3 in normal growth plate chondrocytes. Dvl is a core component of the Wnt/PCP signaling pathway and is required for the regulation of both Wnt/PCP and Wnt/β-catenin signaling. The avidity of the Prickle1:Dvl protein-protein interactions are significantly decreased in the *Prickle1*$^{Bj/Bj}$ cranial base leading to increased Wnt/β-catenin and Wnt/PCP signaling. In the *Prickle1*$^{Bj/Bj}$ synchondroses and growth plates, elevated Wnt/β-catenin results in the precocious maturation and subsequent stalling of terminal differentiation of chondrocytes. Enhanced Wnt/PCP signaling lead to depolarized distribution of Dvl2 and Dvl3 and randomized cell polarity. Our data support the conclusion that Prickle1 and Dvl2 and Dvl3 protein-protein interactions are necessary for normal endochondral ossification.

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Poster #10

Motif dependent Gli-Hand2 synergism is required for mandibular transcriptional networks

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Gli transcription factors (TFs) bind cis-regulatory elements to transduce a Hedgehog (Hh) signal in a context-specific manner, dictating target gene expression and subsequent tissue patterning. Surprisingly, ChIP-seq data revealed that genes associated with the vast majority of genetic loci to which Gli TFs bind in the developing mandible (MNP), are not differentially expressed upon loss of Gli expression. These data suggest additional regulatory inputs are necessary to convey Hh-mediated/Gli-directed tissue patterning. Here, we report that bHLH motifs are among the top-ranked motifs within Gli3 ChIP-seq peaks, and that ChIP-seq peaks for the bHLH TF Hand2, were 111-fold enriched within Gli ChIP-seq peaks. Examination of several target genes revealed Hand2 and Gli3 contribute to gene regulatory networks associated with a variety of critical cell processes of the developing MNP, establishing a mechanism for the micrognathia and hypoglossia observed in Hand2 or Gli conditional knockouts. Furthermore, we observed the lack of canonical (GACCACC), high-affinity Gli binding regions within overlapping Gli3/Hand2 ChIP-seq peaks. Subsequent interrogation of Gli motifs present in Gli3/Hand2 overlapping peaks revealed that Gli3 utilizes a non-canonical, 'divergent' Gli binding motif (GACC*CC) when cooperating with Hand2 which allows for synergistic activation of Gli targets essential for MNP patterning, including *Foxd1*. These data suggest a novel, cooperative mechanism utilized by Gli3 and Hand2 during MNP development. Overall, our work establishes insight into the existence of a sequence-dependent mechanism for propagation of graded Hh signal transduction.

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Poster #11

Investigation of microRNA-149-3p in neural crest cells and its role in orofacial clefting

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Nonsyndromic cleft lip with/without cleft palate (nsCL/P) is a common facial malformation. There is increasing evidence that human neural crest cells (hNCCs), a mesenchymal precursor cell population that gives rise to the majority of cranial cartilage and bones, are major contributors to craniofacial development and disease. NsCL/P has a multifactorial etiology with a strong genetic component. The majority of common associated risk loci map to non-coding regions of the genome. This suggests that the underlying pathomechanisms act through regulatory effects on gene expression, which might be mediated via microRNAs (miRNA).
We first combined array-based miRNA profiling in iPSC-derived hNCC with in-house GWAS data to identify candidate miRNAs for nsCL/P. This resulted in the identification of miR-149-3p as strong candidate, based on consistent hNCC-expression across replicates and the presence of associated risk variants within its genomic region. To investigate the impact of miR-149-3p on neural crest development, we modified miR-149-3p abundance in hiPSC-derived hNCC through overexpression and inhibition. Expression profiling by RNA-Seq revealed differential expression of a number of candidate genes involved in craniofacial development and nsCL/P, such as *BMP7*. Moreover, using scratch assays, we found that the modification of miR-149-3p significantly affected migration of hNCC, which is one of the fundamental properties of hNCC.

Our data suggest that the integration of large-scale genetic data and expression patterns in relevant cell types can help to pinpoint regulatory mechanisms that are involved in craniofacial development and might be related to the etiology of nsCL/P.

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Poster #12

Genes and cell populations involved in facial fusion and clefting

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Facial development is a complex process requiring coordinated growth, movement, fusion and differentiation of distinct facial prominences. Fusion of the facial prominences to form the upper lip and primary palate is a critical part of normal human facial development, and failure of this process results in the most common type of orofacial clefts termed cleft lip with or without cleft palate (CL+/-P). Despite the importance of this process, we only have limited knowledge of the genes and cell populations that are responsible for carrying out fusion of the facial prominences. Here, we employed single cell sequencing to characterize the cell populations associated with patterning and fusion of the upper lip and primary palate. We identified novel ectodermal and mesenchymal cell populations with distinct gene signatures and signals that potentially integrate their behavior during fusion. Furthermore, we recently began to compare how critical new markers of the fusing epithelial seams are altered in several mouse models of human clefting. Our preliminary data indicate various types of pattern dysregulation and we are now chasing the most appropriate model(s) for an in depth single cell sequencing analysis. In conclusion, our studies provide a comprehensive and integrated description of fusion of the upper lip and primary palate at single cell resolution and will provide a crucial understanding for how orofacial clefting occurs at single cell resolution.

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Poster #13

Oriented cell division and spatiotemporally controlled differentiation co-operate to drive anisotropic expansion of bone mesenchyme in the embryonic skull

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A common feature in morphogenesis is anisotropic growth, where tissues do not grow equally in all directions. However, the cellular and physical cues that drive anisotropy in vertebrate tissues are poorly understood, especially in mesenchyme that lack the cell-cell and cell-substrate interactions that instruct morphogenesis in epithelial tissues. Here, we present the embryonic skull cap as a novel model for studying the cellular behaviors driving anisotropic growth in an intact mesenchymal tissue. By tracking osteoblast nuclei *ex vivo,* we find that anisotropic expansion of frontal bones is driven by both intrinsic and extrinsic growth mechanisms. While we find that oriented divisions contribute to intrinsic growth of the bone, these divisions generate anisotropic expansion through biased displacement of daughter cells and limited neighbor exchange. Additionally, and contrary to previous reports, we find extrinsic bone growth that is driven by the progressive differentiation of undifferentiated mesenchyme at the growing edge of the bone. Together, these data suggest that cell displacement and spatiotemporally controlled differentiation co-operate to drive anisotropic bone expansion. Our system is the first live imaging platform to offer subcellular resolution of osteoblasts during skull morphogenesis that could be used to identify fundamental mechanisms regulating shape generation in mesenchymal tissues.

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Poster #14

A mechanistic understanding of genotype-phenotype variation in congenital anomalies

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Genetic and non-genetic modifiers can modulate the outcome of a genotype resulting in phenotypic variance. The variability of genotype-phenotype correlation can be observed in different congenital anomalies such as Treacher-Collins Syndrome (TCS), which is a craniofacial disorder that occurs with an incidence of 1:50,000 live births. TCS is primarily associated with mutations in the *TCOF1* gene, which encodes a nucleolar phosphoprotein called Treacle that is necessary for normal craniofacial development. Currently more than 200 mutations spanning the entire length of Treacle are associated with considerable inter- and intra-familial phenotypic variance in TCS patients, precluding genotype-phenotype correlation. Genetic background and environmental factors may therefore critically influence TCS pathogenesis. It has been established that *Tcof1* haploinsufficiency in mouse models results in deficient ribosome biogenesis and oxidative stress-induced DNA damage, both of which are associated with p53-dependent apoptotic cell death. We discovered that the endogenous levels of Treacle protein as well as reactive oxygen species (ROS) vary according to the genetic background of the mice. Manifestation of the severe phenotype correlates with low levels of Treacle coupled with higher level of endogenous ROS, whereas a mild phenotype correlates with high levels of Treacle and low levels of ROS. Collectively our data provides a novel mechanism to account for the phenotypic variability observed in TCS which could potentially apply to other congenital anomalies.

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Poster #15

Mapping *Loci* associated with skull robustness

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The robustness of a trait to either genetic or environmental variation is a critical feature of biological systems. The study of skull robustness to genetic variation is of special interest, both for describing its genetic architecture and the possible consequences for the skull evolution. Using an advanced intercross population of mice we mapped the genotypic variation associated with the variability of 15 skull traits and identified 53 *loci* associated with the robustness of skull traits (vQTLs), encompassing 1103 genes. Effects that are dependent on either the animal's sex or diet are common, 32 vQTLs have effects on specific subpopulations. The effects of most vQTLs on the skull variability can be described by epistatic interactions, only four vQTLs did not have any significant epistatic interaction. The number of epistatic interactions per vQTL ranged from one to 21 significant interactions and about 20% of these interacting *loci* are actually regular QTLs for skull traits. These results points to a scenario where the genetic control of a trait's variability is highly due to epistatic interactions and a significant proportion of these interactions occur between vQTLs and a regular QTLs. Therefore the evolution of the robustness and the trait itself are linked, selecting for robustness might affect the mean of the trait, and, conversely, selecting on the trait might affect it's robustness. The second is that the models of evolution of robustness should include epistatic interaction, since most, if not all the genotypic variation for trait variability is due to epistasis.

Funding: National Science and Engineering Research Council, Canada (B.H.).

Poster #16

R-spondins from the dental mesenchyme is essential to tooth morphogenesis

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Early development of the teeth requires complex signaling interactions between the mesenchyme and the epithelium mediated by multiple pathways. In particular, the canonical WNT signaling is crucial to odontogenesis, and inhibiting this pathway blocks tooth development at the initiation stage. R-spondins (Rspo) are secreted proteins, and they augment WNT signaling. Although R-spondins were shown to play important roles in development of many organs, their role in tooth development has been unclear. A previous report showed that mutation of Rspo2 in mice led to supernumerary lower molars, while the endogenous teeth developed normally. Given that R-spondin genes have overlapping expression in the face area, it was possible that the relatively mild phenotype of Rspo2 mutant was due to functional compensation by other Rspo proteins. In this study, we have identified Rspo2 and Rspo3 as downstream targets of LHX6 and LHX8 transcription factors in the odontogenic mesenchyme. We found that inactivating Rspo3 in the craniofacial mesenchyme caused loss of lower incisors, which did not progress beyond the bud stage. Simultaneous deletion of Rspo2 and Rspo3 blocked development of all teeth. The incisors were arrested at the early bud stage, while the molars were arrested at the early cap stage. The examination of WNT pathway target Axin2 showed that WNT signaling was impaired in the epithelium more than in the mesenchyme. These results indicate that RSPO2 and RSPO3 mediate the mesenchyme-to-epithelium direction of WNT signaling essential to tooth morphogenesis.

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Poster #17

Analysis of TFAP2A-binding events in a cellular model for nonsyndromic cleft lip +/- palate

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Despite recent success in the identification of underlying genetic risk factors non-syndromic cleft lip with or without cleft palate (nsCL/P), the functional effects of the risk variants are poorly understood. In the present study we hypothesize that allele-specific transcription factor (TF) binding events contribute to a further understanding of the pathobiology of nsCL/P. To investigate this, we established cell culture and ChIP-Seq of human embryonic palatal mesenchyme cells (HEPM), a cell line which has been shown to represent a model for the developing and fusing secondary palate.

Based on 3'RNA-Seq, we identified several TFs that are expressed in HEPM and for which evidence for an involvement in craniofacial development has been presented. We focused our molecular work on TFAP2A that has been shown with a clear role in nsCL/P. Based on ChIP-seq for TFAP2A in HEPM we observed about 7,000 peaks, with motif analysis confirming strong enrichment for the TFAP2A consensus binding within the peaks ($P<2.04\times10^{-4}$). We next identified positional overlaps of our in-house GWAS on nsCL/P, within 865 high-confidence ChiP-Seq peaks. Although none of the 657 SNPs observed within these regions showed genome-wide significance, five SNPs had $P<10^{-3}$ - these might represent risk variants with lower effect sizes. Their genuine association will next be tested in independent replication cohorts, and we will explore preferential allele-specific binding at individual variants. Our strategy will shed light on molecular events that are triggered by associated risk variants, and help to identify regulatory networks and novel risk loci associated with nsCL/P.

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Poster #18

Whole-exome sequencing analysis of multiplex families with nonsyndromic orofacial clefts identifies variants in genes causing Mendelian OFC syndromes

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Orofacial clefts (OFCs) occur when an infants' lip and/or mouth form incorrectly during pregnancy, creating an opening in the mouth. Most OFCs are nonsyndromic, occurring without other structural or developmental abnormalities. Previous research has mostly identified common genetic variants; however, recent analyses have identified rare variants in some cases. We performed whole-exome sequencing (WES) to identify rare variants in 72 multiplex nonsyndromic OFC families with quasi-Mendelian inheritance patterns. We present three families with mutations in genes previously associated with OFC syndromes. The first pedigree consisted of four male siblings with cleft lip and palate (CLP); both parents and a female sibling were unaffected. We identified a frameshift mutation of *TP63* (p.His536Thrfs*18), shared by the affected individuals, the female sibling, and their mother. The second family consisted of two siblings with cleft palate (CP), their father had CLP, and their paternal grandfather had a bifid uvula. They all shared a missense mutation in *IRF6* (p.Leu22Pro). Neither family meets the diagnostic criteria for the syndromes caused by either gene. The third family consisted of two siblings and their father, all affected with CP. We identified a 32bp deletion that results in a frameshift mutation in *SMC3* (p.Leu676Argfs*5) shared among the affected individuals. Mutations in *SMC3* cause Cornelia de Lange syndrome but are primarily missense or in-frame indels, so this variant may cause OFC through a different mechanism. Our study provides evidence that WES can uncover some of the "missing heritability" of OFCs and emphasize the overlapping distinction between syndromic and nonsyndromic OFCs.

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Poster #19

Mapping the Multi-Modal Distribution of Craniofacial Phenotypes in NOSIP Mutants

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Nitric Oxide Synthase Interacting Protein (NOSIP) is a E3 mono-ubiquitin ligase that regulates the activity of protein phosphatase 2A. Deletion of NOSIP leads to severe and variable malformations of the craniofacial complex. Our goal was to use NOSIP mutant mice to understand of mechanisms of phenotypic variation. The phenotype of an individual embryo is the product of the developmental trajectory that it previously traversed. We propose that this concept of canalization is essential for understanding the etiology of complex phenotypic outcomes. We have previously shown that the brain patterns the face, so sequential perturbations affecting the brain and face may create unique developmental trajectories that each embryo transits. We tested this by creating a theoretical 3D morphospace of NOSIP heterozygous and homozygous mutants to determine if embryos occupy unique morphospace regions based on affected craniofacial structures. Embryos between embryonic day 10.5 (E10.5) to E17.5 were collected, fixed, and imaged (□CT). Anatomical features were reconstructed using Avizo. NOISP expression appears to be restricted to the neuroepithelium, lower jaw mesenchyme, and mesenchyme and epithelium in tissue adjacent to the frontonasal ectodermal zone between stage E10.5-11.5. Embryos with a single telencephalic lobe had more severe facial malformations, including micrognathia and obstructed airway, while those with normal telencephalic vesicles had less severe phenotypes, including asymmetry and clefting. Overall, it appears that NOSIP-KO creates a multi-modal distribution of phenotypes along the developmental timeline, suggesting that understanding canalization may provide insight into the complexity of genotype-phenotype map.

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Poster #20

Mutation of mouse *Sf3b4* in neural crest cells model craniofacial abnormalities found in Nager and Rodriguez Syndrome patients.

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Patients with Nager and Rodriguez Syndrome have craniofacial as well as limb abnormalities including downward slanting eyelids, malar and mandibular hypoplasia, cleft palate, as well as radial hypoplasia and thumb abnormalities. Both Syndromes are due to haploinsufficiency of the *SF3B4* gene. *SF3B4* encodes a core component of the U2-major spliceosome complex important for mRNA splicing. We hypothesized that craniofacial abnormalities associated with reduced SF3B4 levels is due to tissue-specific expression and requirement of this gene during embryogenesis. Using whole mount *in situ* hybridization we showed that *Sf3b4* was ubiquitously expressed at early stages of embryogenesis and although still globally expressed at later stages, it was enriched in the maxillomandibular region, limbs and tail bud. To create a mutation in *Sf3b4*, we used CRISPR/Cas9 to target loxP sequences in intronic regions flanking exon 2 and 3 of the *Sf3b4* gene. Deletion of these exons in during embryogenesis after mating with Wnt1-cre transgenic mice revealed hypoplasia of the midbrain and pharyngeal region from E9.5 onwards. Staining for neural crest cells revealed reduced neural crest migration into the pharyngeal region. We will present our characterization of this novel mouse model. Our data suggest that similar to other spliceomopathies, craniofacial malformations in patients with mutation in *SF3B4* are the result of reduced splicing efficiency in neural crest cells.

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Poster #21

Contribution of the neural crest cells to inner ear development

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The stria vascularis (StV) is a specialized epithelial structure of the mammalian cochlea that produces endolymph, the potassium-rich fluid responsible for the positive endocochlear potential. This positive extracellular potential is the major driving force for proper signal transduction by mechanosensory hair cells. The StV is composed of 4 types of cells, marginal cells, Intermediate cells, basal cells and endothelial cells. Intermediate cells of the stria are originated from neural crest cells (NCCs) and are essential for normal hearing. NCCs also give rise to the glial of the VIIIth ganglion and dark cells of the vestibular system. Intermediate cells and the dark cells of the vestibular system are melanocyte-like cells. Mutations that affect NCCs migration often result in syndromes that include deafness. The present challenge is to understand how and when NCCs migrate and integrate into the inner ear. To better understand the contribution of NCCs to the inner ear, we have used transgenic lines to fate map the and separate the temporal contribution of NCCs during the development of inner ear. Our preliminary data suggests that an early migratory population of NCCs give rise to majority of the pigmented and glial cells in the inner ear. During embryonic development the ingression of NCCs into lateral wall of the cochlea follows a basal to apical gradient similar to the gradient of hair cell differentiation.

Poster #22

Ribosomal RNA transcription is tissue-specifically regulated during cranial neural crest development

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Ribsomopathies are tissue-specific congenital disorders that result from disruptions in ribosome biogenesis, a global process essential for cell growth, proliferation and survival. Two examples are Acrofacial Dysostosis-Cincinnati Type (AFDCIN) and Treacher Collins syndrome (TCS), which are primarily defined by anomalies of the head and face. AFDCIN arises from mutations in *POLR1A*, the largest subunit of RNA polymerase I (Pol I), which transcribes ribosomal RNA. TCS is caused by mutations in *TCOF1,* a Pol I-associated factor, and *POLR1C* and *POLR1D*, subunits of Pol I and III. Zebrafish and mouse models of AFDCIN and TCS reveal that *polr1a, polr1c*, *polr1d,* and *Tcof1* loss-of-function leads to reduced rRNA transcription by Pol I, which is a rate-limiting step of ribosome biogenesis. This results in p53-dependent neuroepithelial cell death and reduced proliferation of neural crest cells (NCC), which leads to cranioskeletal defects. To understand the function of ribosome biogenesis specifically in NCC, we conditionally deleted *Polr1a, Polr1c,* and *Tcof1* from NCC in mice. Our data shows that *Polr1a*, *Polr1c,* and *Tcof1* are essential for embryonic survival and that these genes play a specific role in rRNA transcription during NCC-derived cranioskeletal development. Additionally, we show that rRNA is differentially regulated and that NCC are highly susceptible to disruptions in rRNA transcription. Overall, our work provides novel insights into the tissue-specific roles of ribosome biogenesis in NCC and cranioskeletal development. Furthermore, our work will aid in identifying mechanisms underlying the pathogenesis and possible prevention of AFDCIN, TCS and other ribosomopathies and neurocristopathies.

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Poster #23

Facial shape and allometry QTL in the Diversity Outbred mouse implicate genes critical to skull and skeletal development

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Discovering the genes that contribute to differences in facial morphology is one key to untangling how faces are built and come to differ from one another. This study maps quantitative trait loci (QTL) for skeletal facial shape using Diversity Outbred (DO) mice. The DO is a randomly outcrossed population with high heterozygosity that captures the allelic diversity of eight inbred mouse lines from three subspecies. The study uses a sample of 1147 DO animals, each characterized by 22 three-dimensional landmarks, 56,885 autosomal and X-chromosome markers, and sex and age classifiers. Quantifying marker effects with a mixed effects regression that accounts for kinship among subjects resulted in identification of 37 facial shape QTL across 20 shape principal components (PCs). These include some previously identified intervals as well as new regions that expand the list of potential targets for further experimental study. Three QTL characterize shape associations with size (allometry). Median support interval size is 3.5 Mb. Narrowing additional analysis to QTL for the five largest magnitude shape PCs, we find significant overrepresentation of genes with known roles in growth and skeletal and sensory organ development. For each interval, at least one of these genes tends to lie within 0.25 Mb of the QTL's peak. QTL effect sizes are small, with none explaining more than 0.5% of facial shape variation. Thus, our results are consistent with a model of facial diversity that is influenced by key genes in skeletal and facial development and, simultaneously, highly polygenic.

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Poster #24

Arhgap29 in oral epithelial cells is required for proper palatogenesis

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ARHGAP29 belongs to the family of small GTPase activating proteins that negatively regulate the activity of RhoA in cells. In humans, genetic variants in *ARHGAP29* have been associated with increased risk for nonsyndromic cleft lip and/or palate (NSCL/P). In mice, *Arhgap29* is necessary for viability as homozygote nulls die prior to embryonic day (e) 8.5, prohibiting understanding its function in craniofacial development. Therefore, the rationale for this study was to carry out experiments with conditional loss of *Arhgap29* to ascertain its role during craniofacial development. We used a novel Arhgap29 floxed allele (*A29$_{fl}$*) in combination with a Keratin 14 Cre recombinase driver (K14Cre) to delete Arhgap29 from ectodermal cells, including the oral epithelium and the epidermis. At e14.5, genotypes were distributed in proper Mendelian ratios and K14Cre;A29$_{fl}$ animals survived into adulthood. To determine the role of Arhgap29 in palatogenesis, we performed histological examination of coronal sections of eight e14.5 litters. Our results show a significant increase in the number of embryos with cleft palate in K14Cre;A29$_{fl}$ compared to wild type embryos. While we hypothesized that *Arhgap29* conditional null mutants would exhibit cleft palate due to abnormal oral adhesions as we previously described for a heterozygous loss of function *Arhgap29* mouse model, this was not the case in the K14Cre;A29$_{fl,}$ but rather delay in elevation and migration of the palatal shelves. These data show that *Arhgap29* in oral epithelial cells contributes to palatogenesis, a previously undescribed role for this protein.

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Poster # 25

Defining Ectodermal Cell Dynamics During Mouth Opening in Developing Zebrafish

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The mouth connects the outside world to the internal environment, and is present in all animals. A key step in the formation of the mouth is controlled breach of the ectodermal body covering. However, the sequence of cellular morphogenetic events in the ectoderm that lead to the opening of the mouth is not well understood due to a lack of *in vivo* imaging tools to capture this dynamic process. Here we use high-resolution *in vivo* timelapse imaging to visualize the cellular events during orofacial morphogenesis in the developing zebrafish. To determine the role of ectoderm morphogenesis in the formation of the mouth, we utilized a set of GAL4 enhancer trap lines that are expressed in discrete epithelial cell types in the face and mouth of the developing zebrafish. When combined with UAS effector lines, these epithelial GAL4 lines provide the opportunity to visualize specific cells for imaging and target cells for ablation. We tracked different ectodermal cell types, both the periderm and p63-positive basal cells, in real time throughout formation of the mouth. Using this approach**,** we show distinct cellular movements and behaviors during the opening of the mouth. We then ablated the different ectodermal cell types early in development and quantified the resulting morphological changes to mouth formation. Unbiased morphometric analysis using automated measurements of facial structures revealed a significant change in the opening of the mouth and morphology of the oral cavity after ectoderm perturbation. Intriguingly, we also observed restoration of normal morphology later in development, suggesting a robust regenerative response to ensure mouth opening and formation. Together, this study provides a high-resolution *in vivo* characterization of the cellular mechanisms regulating ectoderm morphogenesis during formation of the mouth and oral cavity.

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Poster #26

Exome Sequencing identify a Damaging Mutation in ABI3BP in a Family with Hereditary Gingival Fibromatosis from Nigeria

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Hereditary gingival fibromatosis (HGF) is a slow and progressive enlargement of the attached gingiva covering the teeth and affecting speech, mastication and aesthetics. This is a rare oral disease which may be localized or generalized, may be isolated or part of a syndrome. Several genes have been associated with the syndromic type however little is known about the genetic factors associated with the isolated type. In identifying the variants that may be associated with isolated form, an Whole exome sequencing of DNA samples from an affected family was done. VarSeq® software was used in the annotation and analyses of the variants called. The called variants were filtered for shared dominant variants (present only in the father and son but absent in the mother). These variants were also filtered for very low minor allele frequency by comparing to the publicly available control database which contains over 5000 individuals from African populations. In silico tools like Polyphen, SIFT, HOPE and CADD scores were used to predict the effects of the variants. Analyses of the 207,026 variants that passed the quality check resulted in 10 variants which were validated by Sanger sequencing. Among the 10 variants, the ABI3BP variant (c.53G>T p.Cys18Tyr) is predicted to be deleterious and damaging by SIFT and PolyPhen, pathogenic by ClinVar and among the top 1% deleterious mutation in the human genome. This gene has 2 fibronectin domains and it is involved in collagen binding and organization of the extracellular matrix.

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Poster #27

Analysis of zebrafish periderm enhancers illuminates the periderm gene regulatory network and facilitates identification of an orofacial-cleft-associated regulatory variant near *KRT18*.

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Genome wide association studies for non-syndromic orofacial cleft (OFC) have identified several loci where the presumed risk-relevant gene is expressed in the superficial (periderm) layer oral epithelium. The single nucleotide polymorphisms (SNPs) at such loci that directly influence risk for OFC are unknown but likely disrupt key transcription factor binding sites within periderm enhancers. Tissue-specific enhancers in zebrafish are often enriched for the same binding sites as their human counterparts. Therefore we isolated periderm cells from zebrafish embryos by sorting, carried out ATAC-seq on both selected and flow-through cells, and integrated periderm-specific ATAC-seq peaks with H3K27Ac peaks from whole embryos. Elements thus identified had periderm enhancer activity in reporter assays *in vivo*. Sequence motifs enriched in these elements were found to be essential for this activity and corresponded to binding sites of known and novel transcriptional regulators of periderm differentiation. We similarly identified sets of enhancers that are active in mouse embryonic palate epithelium and in a human oral epithelium cell line. Gapped-kmer-support-vector-machine (SVM) classifiers were trained on these sets and used to rank OFC-risk-associated SNPs near *KRT18*, a gene expressed in periderm, for their effects on the scores of the encompassing elements (deltaSVM). Interestingly, all the classifiers assigned the strongest deltaSVM to the same SNP, but the significance was strongest when the classifier was trained on periderm enhancers. Reporter assays confirmed that this SNP affects the strength of an epithelial enhancer. These findings are relevant to interpreting rare and common variants associated with risk for orofacial clefting.

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Poster #28

A large-scale genome-wide association scan in East Asians revealed genetic mechanisms underlying variations of human facial morphology

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The facial surface is visible and recognizable based on the facial shape variation which represents a set of highly polygenic and correlated complex traits. Understanding the genetic basis underlying facial shape traits has important implications in population genetics, developmental biology, and forensic science. However, relatively little is known about which and how genes influence human facial variation in East Asians. Here, we conduct a genome-wide association scan for facial features in ~10,500 East Asians from high resolution 3dMD facial images. We identified 109 loci that significantly associated with facial shape at multiple levels of organization, showing distinctive patterns of global-to-local genetic effects on facial shape. Among the 109 loci, 31 were supported in the pre-facial GWAS literature, while 78 were newly identified. Functional enrichment analysis showed that most of genes were linked to skeletal system development, regionalization, pattern specification process and embryonic organ development, suggesting that the facial morphology variation be shaped during the pattern specification process. Finally, we found that the associated loci were enriched for signals of positive selection, indicating that facial morphology had undergone strong local adaptation during the recent evolutionary history. These results substantially advanced our understanding of the genetic mechanisms underlying human facial variations.

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Poster #29

Alx4 and Six2 act synergistically to control frontonasal and palate development

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Frontonasal dysplasias (FND) are well-documented congenital craniofacial malformations characterized by hypertelorism, broad or cleft nose with abnormal nasal tip, frontal bossing, and sometimes cleft lip and palate. Whilst most FND cases occur sporadically with unknown etiology, homozygous loss-of-function mutations in the *ALX* family genes, including *ALX1*, *ALX3*, and *ALX4*, have been identified as the genetic causes for autosomal recessive FND, with disruption of *ALX1* associated with severe facial clefting while mutations in *ALX3* and *ALX4* resulted in milder but clinically distinctive frontonasal malformations. However, how the ALX transcription factors regulate craniofacial development is not well understood and the molecular mechanisms underlying FND pathogenesis remain largely unresolved. Recently, two reports have identified association of heterozygous deletions of the *SIX2* gene with a mild form of FND. *Six2* exhibits partly overlapping expression with the *Alx* genes during frontonasal and palate development in mouse embryos. Whereas mice lacking either *Six2* or *Alx4* do not have apparent defect in frontonasal development, we found that *Alx4*$^{-/-}$*Six2*$^{-/-}$ mutant pups exhibit severe midfacial clefting. In addition, *Alx4* and *Six2* exhibit dose-dependent genetic interactions in palatogenesis, with 62% of *Alx4*$^{+/-}$*Six2*$^{-/-}$ and 100% of *Alx4*$^{-/-}$*Six2*$^{-/-}$ compound mutant pups exhibiting cleft palate. These results indicate that Six2 function synergistically with Alx4 to regulate frontonasal and palate development.

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Poster #30

HCFC1: A new transcriptional regulator coupling vitamin B12 metabolism to neural crest development

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Recently, patients with cblX, an X-linked variant of the cblC cobalamin (vitamin B12) deficiency syndrome, were discovered. While cblX phenocopies most of the clinical symptoms observed in cblC, the disease is caused by mutations in the transcription cofactor *HCFC1*. Previous research has determined that HCFC1, along with the transcription factor RONIN, directly regulates the expression of *MMACHC*, which encodes an enzyme essential for cobalamin metabolism and is mutated in cblC patients. Thus, the origin of the cblX phenotype is likely in part due to transcriptional reduction of *MMACHC* during development.

To address this possibility and define the exact pathophysiology of cblX, our group has generated the *Hcfc1 A115V* mouse model, which carries the most frequent missense mutation observed in cblX patients. Besides craniofacial dysmorphia as found in the cblX patients, the *A115V* hemizygous mice also exhibit a fully penetrant hypopigmentation phenotype, indicating a potential defect in neural crest cell (NCC) development. These data have led us to hypothesize that HCFC1 functions in a transcription factor complex to regulate NCC development through regulating *Mmachc* and cobalamin metabolism.

We have determined that, analogous to cblX patients, *A115V* hemizygous mice have a dramatic reduction of *Mmachc* levels. Moreover, the hypopigmentation phenotype is rescued by over-expressing *Mmachc* in these mice. These data suggest that we have uncovered a new transcriptional program influencing NCC development and raises the intriguing possibility that disrupted cobalamin metabolism may also play a role. Current efforts are aimed at determining the specific requirement for cobalamin during craniofacial and NCC development.

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Poster #31

Proteomic characterization of the inner ear in *Xenopus laevis*

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Hearing loss is one of the most prevalent birth defects in which 1.4 in 1000 infants screened in the US have hearing loss. To understand the molecular mechanisms that lead to congenital hearing loss, we need to expand our current knowledge of the proteins that play important roles in forming the inner ear during development. Recent studies in *Xenopus* have explored transcriptomic profiles of inner ears to reveal human hearing loss gene orthologs. However, the information on the numerous proteins and their dynamics during otic formation is still lacking. This is important because often during developmental processes, levels of mRNAs do not predict protein levels. To address this knowledge gap, we used liquid chromatography and high-resolution mass spectrometry to quantify otic proteins over the course of otic morphogenesis. Inner ears at different stages of otic patterning were dissected from larvae/tadpoles. Proteins were extracted and digested with trypsin. The resulting peptides from each stage were barcoded with tandem mass tags and analyzed to quantify relative proteomic changes between different stages of otic development. Our preliminary analysis enabled identification of >3,000 protein groups including ~74 proteins associated with deafness in humans. We are now exploring this information to assess proteome remodeling during IE development and disease.

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Poster #32

SPECC1L-deficient primary palate mesenchyme cells show impaired collective cell migration attributes that are rescued by upregulation of PI3K-AKT pathway

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Clefts of the lip and/or palate (CL/P) are common anomalies that occur in 1/700 live-births. Patients with pathogenic *SPECC1L* variants show a range of craniofacial defects including hypertelorism and CL/P. *Specc1l* null mutants were embryonic lethal with defective neural tube closure. Hypomorphic *Specc1l*$_{\Delta C510}$ truncation mutants died perinatally, but did not show cleft palate. *Specc1l*$_{null/\Delta C510}$ compound mutants resulted in transient oral adhesions, subepidermal blebbing, and delayed palate elevation. Palate elevation requires extensive remodeling of the neural-crest-derived palate mesenchymal cells, which we posit involves collective directional cell movement (CCM). We performed live time-lapse microscopy of wildtype and SPECC1L-deficient primary mouse embryo palatal mesenchyme (MEPM) cells in open-field and wound-healing assays. Wildtype MEPMs indeed showed cell-stream formation – a CCM attribute – and these streams were consistently narrower for SPECC1L-deficient cells. SPECC1L-deficient MEPM cells also showed delayed wound-healing. Movement *parallel* to the direction of wound-front propagation can be measured as an attribute of CCM. Trajectories of SPECC1L-deficient cells showed increased movement *perpendicular* to the direction of wound-healing. In the presence of adequate motility, this diminished directionality likely caused delayed wound-healing. We previously reported reduced PI3K-AKT signaling upon SPECC1L deficiency. Consistently, activation of the PI3K-AKT pathway using 740-Y-P small-molecule activator rescued wound-healing delay, and the underlying CCM defect, in SPECC1L-deficient MEPM cells. Our data are the first to show CCM attributes in MEPM cells, and to propose the use of MEPM cells to study mesenchymal remodeling during palatal shelf elevation. We also show a novel role for SPECC1L in CCM through modulation of P13K-AKT signaling.

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Poster #33

Validating genetic variants of Auriculocondylar Syndrome Type 2 using cell signaling reporters and CRISPR mice.

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Auriculocondylar Syndrome (ARCND) is a rare craniofacial disorder defined by mandibular hypoplasia, temporal-mandibular joint (TMJ) fusion, and question mark ears. Genetic variants in effectors of endothelin receptor type A (EDNRA) have been implicated in ARCND, but there is little evidence they are directly responsible for perturbed signaling and craniofacial defects. To address this deficit, we tested the hypothesis that ARCND Type 2 alleles, which are missense variants in *PLCB4,* produce pathogenic dominant negative (DN) variants of phospholipase C β4 (PLCβ4), an EDNRA signaling effector. Using a Bioluminescence Resonance Energy Transfer assay that measures PLCβ activity, we observed that overexpression of ARCND variants R621H or Y623C abrogated EDN1-mediated activation of wildtype PLCβ4. In conjunction, we generated G0 mice harboring the R621H variant using a CRISPR-based homology-directed repair (HDR) targeting strategy to determine whether this *PLCB4* variant is sufficient to recapitulate ARCND phenotypes. Of 61 E18.5 G0 embryos generated, 10 contained either HDR (n=3) or non-homologous end joining (NHEJ, n=5) in *PLCB4*. Of the five embryos examined thus far, four had unilateral defects in the proximal mandible and ectopic bone struts that extended from the middle ear to the skull base. Similar bones have been observed in mouse embryos in which EDNRA signaling is reduced but not lost, indicating that EDNRA signaling is partially disrupted in these CRISPR mice. Our results illustrate a relatively rapid workflow for testing the genotype-phenotype relationship for disease-associated alleles while at the same time illustrating how disease models can inform analysis of humans with facial birth defects.

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Poster #34

Web-based delivery of cross reality (XR) medical images for collaborative craniofacial disease assessment.

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XR visualization facilitates exploration of data sets for the purpose of diagnostic assessment. The purpose of this study was to develop a web-based delivery system for collaborative disease assessment based on craniofacial imaging utilizing XR visualization. The client-server model was designed as a cloud-based structure via Architecture as Code (AoC) enabling hardware infrastructure to be specified by version controlled configuration files. The software server code comprises micro-service containers providing security and fault-tolerance by segregating code execution. This micro-service system orchestrates the distribution of WebSocket messages to facilitate collaboration between users. Delivery is achieved through a web embedded volumetric renderer written in javascript using hardware agnostic WebGL2 specifications. As an application, a 64-year old Pacific Islander presented with a history of stage III cancer, right-kidney nephrectomy, DMT2 and a BMI of 28.7. A CBCT scan series was performed (i-CAT 3D Imaging Solution, 74 kVp and 16 mA,16 second exposure) and consultation was performed remotely. Portions of calcified carotid arteries and branches appeared as straight, parallel pairs of radiopaque lines with a ring-like pattern in cross section. Most of the branches of the third part of the maxillary artery were affected including the infraorbital and sphenopalatine arteries. A portion of the descending palatine was also calcified. The diagnosis was Mönckeberg's sclerosis and this is the first report of carotid artery involvement in this syndrome. Results of this study demonstrate the utility of a web-based XR system applied to remote consultation for the diagnosis of rare craniofacial disease.

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Poster #35

A genome-wide association study on three-dimensional (3D) skull phenotypes in Han Chinese population

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As an important sign of identity recognition, human craniofacial morphology involves complex traits of high heritability. However, the genetic basis of human normal craniofacial phenotypes is largely unknown. Currently, most of genome-wide association studies (GWAS) on facial morphology are based on 2D/3D facial images which captured the overlying soft tissue facial structures. Such phenotyping approach is vulnerable to changes in muscles and fat, leading to difficulty of reproducibility. In contrast, we performed a GWAS on 3D skull phenotypes using head CT images of Han Chinese population. In our initial discovery panel, cranial CT images of nearly 800 individuals were objectively quantified by image preprocessing, skull segmentation and 3D reconstruction. We developed automated localization of 21 anatomical landmarks on the skull using multi-atlas registration on 30 training atlases. Then, we extracted 18 predefined landmark-measured phenotypes and 153 pairwise landmark distances followed by principle component analysis. Further, we analyzed 3D shape-related craniofacial phenotypes and carried out the GWAS using Illumina GSA 700K SNP Array with imputed genotypes. We discovered a novel candidate region of CTCF binding site located on chromosome 12q13.1 that significantly affected skull sphericity and a combined-GWAS analysis showed a potential signal on chromosome 15q22.3 associated with skull flatness. A larger sample size will be needed to validate and detect more candidate regions that affect human craniofacial morphology in the future. To our knowledge, our study has brought new insight into the genetic basis of craniofacial variations in Han Chinese population and will largely complement current human craniofacial genetics.

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Poster #36

Identifying genetic architecture of facial morphology in Uyghurs

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Human facial morphogenesis is generated synthetically by craniofacial, skin and muscle developmental processes. Twin studies found that the heritability of facial features is around 80%. In this study, we conducted a genome-wide association analysis of facial morphological phenotypes measured from 3D facial images of 3,000 Uyghur individuals. Based on seventeen landmarks of facial 3D images, we used four schemes to efficiently characterize the facial morphological variations: traditional inter-landmark distances, geometric measurements of landmarks, principal components of clustered inter-landmark distances, and partial generalized procrustes analysis of landmarks. We identified ten genome-wide significant SNPs of seven genes: DRD1, CNBD1, SBF2, SOX9, ALG10B, EDAR and CASC17. In particular, deficiency of SOX9 can lead to the skeletal malformation syndrome, campomelic dysplasia and cleft palate. In addition, we also found that several pleiotropic SNPs, e.g., rs3827760 in the exonic region of EDAR, are associated with facial and multiple other phenotypes. Our study thus provides some insights into the genetic mechanisms of facial morphogenesis.

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Poster #37

Reduced size and complexity of the mandibular first molar in *Fgfr3*$^{P244R/P244R}$ mice

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Muenke syndrome is the most common human craniosynostosis syndrome, characterized by coronal suture synostosis, hearing loss, developmental delay, carpal and tarsal fusions and the Pro250Arg mutation in the FGFR3. Less is known about the effect of this mutation on tooth development. Furthering our understanding of the role of FGFR3 in molar morphogenesis can shed light on the evolutionary mechanisms underlying morphological change. We evaluated the role of the FGFR3 Pro250Arg mutation in the morphogenesis of the left lower first molar (M_1) in mice at P10 with the analogous mouse FGFR3 Pro244Arg mutation. µCT scans of mice heterozygous (*Fgfr3*$^{P244R/+}$; N=15), homozygous (*Fgfr3*$^{P244R/P244R}$; N=10), and unaffected littermates (*Fgfr3*$^{+/+}$; N=12) were used to create 3D surface reconstructions of the unerupted, but fully formed, M_1 enamel caps. These 3D surfaces were used to measure the size, surface curvature, undulation, and complexity, and to assess crest formation. Molar size was quantified using linear measures (length and width) and crest formation was scored qualitatively. Surface curvature, undulation, and complexity were assessed using quantitative topographic analyses including Dirichlet Normal Energy, Relief Index, and Orientation Patch Count. Results indicate that the first mandibular molars of *Fgfr3*$^{P244R/P244R}$ mice are relatively smaller and less complex, with a mesial cusp that is oriented differently than that of *Fgfr3*$^{+/+}$ mice. Additionally, the M_1 of *Fgfr3*$^{P244R/P244R}$ mice lacks a mesiobuccal cusp that is present in *Fgfr3*$^{+/+}$ specimens. The results also indicate that relative to *Fgfr3*$^{+/+}$ and *Fgfr3*$^{P244R/+}$ specimens, *Fgfr3*$^{P244R/P244R}$ specimens exhibit less variation in surface curvature, undulation, and complexity.

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Poster #38

BMP signaling in cranial bone development and craniosynostosis

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A subset of craniosynostosis (CS) cases are due to single mutations, but in most the genetic risk is more complex. Human genetics strongly suggest that enhanced BMP signaling increases CS risk. A recent association study for CS identified a risk locus in the *BBS9* gene, but without associated coding sequence changes. The adjacent gene encodes Bmper, an extracellular modulator of BMP signaling. In zebrafish, *bmper* is a prominent marker for differentiating osteoblasts, but its role in regulating BMP signaling in bone is unclear. We hypothesize that Bmper enhances BMP signaling during skull development, and that changes in a distal enhancer of *BMPER* actually underlie the genetic risk of CS. We are using transgenic zebrafish to screen for human *BMPER* enhancers, in part to identify candidate sequences for CS risk. We have found multiple enhancers in the interval encompassing *BBS9* and *BMPER* that direct expression to skeletal tissues during craniofacial development. We have used sequential live confocal microscopy to describe dynamic enhancer activity during skull formation. We are also examining their activity in *sp7* mutants, which have dramatically increased BMP signaling. To determine which cells show upregulation of BMP pathway components, and how lineage and differentiation of osteoblasts change in the absence of *sp7*, we subjected cells of wildtype and mutant skulls to single cell RNA sequencing. Our efforts to understand the transcriptional regulation of *BMPER*, and the specific role of BMP signaling at the sutures, are aimed at revealing the mechanism underlying a major genetic risk factor for CS.

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Poster #39

Regulation of cranial neural crest cell fate by a dual-transcription factor model

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A wide variety of common craniofacial birth defects occurs due to errors in the development of cranial neural crest cells (CNCCs) that give rise to craniofacial skeletal and peripheral nervous systems. Identification of the genetic and environmental factors that regulate cell fate of CNCCs is crucial for uncovering the causes behind craniofacial disorders. Our lab has identified a dual-transcription factor pathway that regulates CNCCs during EMT and migration. Initially, we identified a causative mutation in a TWIST1 binding site within an *Interferon Regulatory Factor 6* (*IRF6*) enhancer in a multi-generational family affected with syndromic cleft lip and palate. Our subsequent animal work has shown that *Twist1* and *Irf6* genetically interact during early craniofacial development, and that the compound heterozygotes exhibit craniofacial abnormalities. We recently demonstrated that *Irf6* is required for neural tube and CNCC development and that it interacts with *Twist1* during EMT and migration of CNCCs. We also showed that TWIST1 is highly phosphorylated in CNCCs and its phosphorylation is crucial for regulating *Irf6* and potentially *miR10* family members in CNCC-derived craniofacial tissues. Our recent *in-vivo* and neural tube explants using dual fluorescent cell tracing system showed that *Twist1* conditional knockout (CKO) in CNCCs significantly reduced cell delamination and disrupted EMT. The detached *Twist1* CKO in CNCCs retained their epithelial signatures and migrated as clusters of epithelial-like cells over significantly shorter distances. Finally, *Twist1* phospho-incompetent mice for two serine residues were recently generated to determine their impact on TWIST1 activity. Our preliminary results showed that *Twist1*$^{S68A/S68A}$ phospho-mutant mice have craniofacial bone defects and forebrain hemorrhage.

Poster #40

Contribution of Rare Variants in the Noncanonical Wnt/Planar Cell Polarity Pathway Genes to Nonsyndromic Cleft Lip and/or Palate.

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Nonsyndromic cleft lip/palate (NSCL/P) is a complex trait, influenced by low-effect common variants and high-effect rare variants, as those in Wnt/b-catenin pathway (e.g., *CDH1*, *CTNND1*), mostly detected in familial cases (which comprise 30% of NSCL/P). In contrast, noncanonycal Wnt/Planar Cell Polarity (PCP) pathway, which plays an important role in vertebrate craniofacial development, has been underexplored in NSCL/P. We aimed to determine the contribution of rare variants in Wnt/PCP genes to NSCL/P. By sequencing 9 PCP genes in 251 probands of familial NSCL/P, we found 27 rare missense variants with CADD>20 in *CELSR3*, *DVL3*, *FZD7*, *PRICKLE1*, *VANGL2*, *WNT5A* and *WNT11*, and one private heterozygous frameshift deletion in *PRICKLE1,* a highly intolerant gene to loss-of-function variants. No aggregation of rare variants was observed for any gene, comparing with Brazilian controls, although *PRICKLE1* (P=0.05) and *WNT11* (P=0.06) reached borderline significance. The frameshift variant in *PRICKLE1* segregates in proband's affected mother and brother; additional candidate variant or copy-number variants in this family were not found by exome and array-CGH analysis. Although our data suggest that rare variants in core Wnt/PCP genes are not a prevalent cause of NSCL/P, the frameshift variant in *PRICKLE1* may be causal. *PRICKLE1*'s role in NSCL/P etiology has been supported by mouse mutants, and *PRICKLE1* has also been shown to regulate levels of cdh1 prior to NCC delamination in zebrafish. Our results may represent the first case of a *PRICKLE1* pathogenic variant in familial NSCL/P. Ongoing functional cellular studies will provide further insights into variant's pathogenicity and etiological mechanism involved.

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Poster #41

The influence of individual EphB receptors on facial shape

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Efnb1$^{+/-}$ heterozygous and *Efnb1*$^{-/Y}$ hemizygous mice phenocopy many aspects of craniofrontonasal syndrome (CFNS), a syndrome caused by EFNB1 loss of function mutations. At E14.5, they display hypertelorism, facial shortening, and relatively inferior nostrils and ear pinnae, with *Efnb1*$^{+/-}$ displaying greater severity of each phenotype. EphB1, EphB2, and EphB3 are the principle receptors for ephrin-B1, but it is unknown which receptors are relevant to which CFNS phenotypes and whether global additive or distinct tissue-specific functions are conferred by each receptor. We completed morphometric analysis of µCT images of an E14.5 allelic series of compound *Ephb1; Ephb2; Ephb3* null mutants to assess the contributions of each receptor to craniofacial morphogenesis. Procrustes ANOVA analysis indicates that facial size and each receptor contributes significantly to facial shape, although *Ephb1* accounts for very little shape variance. We accounted for the effect of facial size on shape (allometry) to clarify individual receptor effects. The additive effect of the *Ephb1* null allele is almost unnoticeable, suggesting only minor interactions between *Efnb1* and *Ephb1* during facial morphogenesis. *Ephb2* and *Ephb3* loss of function both lead to hypertelorism, increased whisker region height, and inferior nostrils; with increased severity when function of both receptors is lost. Although *Ephb2* null mutants consistently exhibit dysmorphology regardless of the genotype of other receptors, *Ephb3* loss leads to effects across more of the face, including at the ear pinnae and lip margins. *Ephb2* and *Ephb3* receptor loss of function leads to overlapping, but distinct dysmorphology, suggesting both are important for maintaining normal facial morphology.

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Poster #42

Integrated transcriptome and network analyses reveal spatiotemporal dynamics in dysmorphic skull development

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Craniofacial dysostosis, including craniosynostosis, is a common birth defect that occurs in one in 2,000 newborns. The metopic suture, one of the major craniofacial sutures, is prematurely fused in 25% of all craniosynostosis cases. This suture is abnormally widened in Apert and Saethre-Chotzen craniosynostosis syndromes. No more than 30 genes and few mouse models are known to be associated with metopic abnormalities. To characterize the transcriptome of the murine frontal suture (homologous to the human metopic suture) during embryonic development in mutants compared to wildtype (WT), we generated bulk and single-cell RNA-seq data and performed multiscale network analysis. Bulk RNA-seq analysis was performed on laser capture microdissected tissue of 60 suture mesenchyme (SM) and osteogenic front (OF) subregions at embryonic day (E)16.5 and E18.5 of Apert *Fgfr2*$^{+/S252W}$ and Saethre-Chotzen *Twist1*$^{+/-}$ mutants, with widened frontal sutures at E18.5, and WT mice. Across both subregions, there were 2,480 differentially expressed genes (DEGs) between E16.5 and E18.5. In the *Twist1*$^{+/-}$ and *Fgfr2*$^{+/S252W}$ mice across both ages, there were 115 and 127 DEGs, respectively. Transcriptional changes affecting genes involved in vasculogenesis and ribogenesis distinguished the *Twist1*$^{+/-}$ and *Fgfr2*$^{+/S252W}$ mice from WT, respectively. Co-expression network analysis characterizing the transcriptional organization of the SM and OFs identified a novel SM gene expression module, from which we validated key driver genes regulating osteoblast differentiation. Single-cell RNA-seq analysis identified at least five suture subpopulations that suggested an osteogenic differentiation trajectory within the suture. Altogether, our rich transcriptomic data with network biology analyses provided a model of frontal suturogenesis.

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Poster #43

Requirement of hyaluronan synthase-2 in craniofacial and palate development

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Whereas it has been widely accepted that accumulation of hyaluronan in developing palatal mesenchyme plays a major role in palatal shelf elevation, direct genetic evidence for a requirement of hyaluronan in palatal shelf elevation is lacking. We show that Has2, one of three hyaluronan synthases in mammals, plays a major role in hyaluronan synthesis in the neural crest-derived palatal mesenchyme. Inactivation of *Has2* throughout the neural crest lineage using the *Wnt1-Cre* driver or specifically in developing palatal mesenchyme using the *Osr2-Cre* driver caused reduced palatal shelf size and increased palatal mesenchyme cell density prior to the time of normal palatal shelf elevation. However, whereas *Has2$^{f/f}$;Wnt1-Cre* fetuses showed dramatically reduced mandible size and complete failure of palatal shelf elevation, *Has2$^{f/f}$;Osr2-Cre* fetuses showed delayed palatal shelf elevation. Furthermore, tissue-specific inactivation of *Has2* in the mandibular arch mesenchyme using the *Hand2-Cre* driver, caused mandibular malformation and cleft palate associated with disruption of palatal shelf elevation. Explant culture assays indicate that disruption of palatal shelf elevation in *Has2$^{f/f}$;Hand2-Cre* fetuses resulted from physical obstruction by the malformed mandible and tongue. Together, these data indicate that hyaluronan plays a crucial intrinsic role in palatal shelf expansion and timely reorientation to the horizontal position above the tongue as well as an important role in mandibular morphogenesis that secondarily affects palatal shelf elevation.

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Poster #44

P-bodies mediate the post-transcriptional regulation of *Draxin* during cranial neural crest EMT

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Neural crest cells undergo a tightly regulated epithelial-to-mesenchymal transition (EMT) to delaminate from the neural tube. In the cranial neural crest, we have shown that this developmental EMT program is controlled by temporally restricted expression of the Wnt antagonist, Draxin. A hallmark of Draxin's function during cranial neural crest EMT is its transient expression. However, precisely how *Draxin* expression is regulated has been unclear. Here, we show with an *in vivo* reporter construct that the rapid degradation of *Draxin* mRNA is mediated post-transcriptionally via its 3'-untranslated region (3'-UTR). Using an MS2-MCP reporter system and time lapse imaging of cranial neural crest explants, we further demonstrate that the MS2 construct containing the 3'-UTR of *Draxin* (MS2-*Draxin* 3'-UTR) localizes to small cytoplasmic granules resembling P-bodies in migrating cranial neural crest cells. To further characterize these granules, we performed *in situ* hybridization for known P-body components, and found that P-body markers *CNOT1*, *EDC3/4*, and *TNRC6A* (GW182) mRNAs are expressed in migratory cranial neural crest. Using perturbation of P-bodies with both cycloheximide, and mutant forms of GW182 and DDX6, we describe the role of P-bodies during cranial neural crest migration and in the degradation of *Draxin* during cranial neural crest EMT. Together, our data highlight a novel and important role for P-bodies in an intact organismal system— playing an essential role in cranial neural crest EMT via post-transcriptional target degradation.

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Poster #45

Novel Pathogenic Variants Support Constitutive Dysregulation of the Noncanonical WNT Pathway Gives Rise to Robinow Syndrome

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Robinow syndrome (RS) is a genetically heterogeneous disorder characterized by skeletal dysplasia and distinctive facial appearances. Previous studies have revealed locus heterogeneity and Robinow genes identified thus far all play a role in WNT/PCP signaling. We performed exome sequencing (ES) on a cohort of 13 with RS or RS-like phenotypes. Two individuals had pathogenic variants in *DVL1*: a recurrent frameshifting variant (c.1519delT; Trp507Glyfs*142), and another at c.1556del (p.Gly519Aspfs*130). Two subjects had pathogenic variants in *FZD2*: a missense at c.1300G>A (p.Gly434Ser) and a nonsense at c.1644G>A (p.Trp548*). In addition, three individuals carried likely pathogenic variants in *WNT5A*: a missense (c.461G>T; p.Cys154Phe) was found in a patient and his affected father, and a missense (c.248G>A; p.Cys83Tyr) in an unrelated individual. One subject had variants in *NXN*, including a nonsense (c.817G>A; p.Gln273*) *in trans* with a ~1Mb telomeric deletion (GRCh38/hg38 17p13.3(chr17:0-1123461)x1). Clinical findings in subjects diagnosed with RS can elicit a range of potential differential diagnoses. In our cohort, pathogenic frameshift variants causative of Aarskog-Scott syndrome (MIM: 305400) were found in four individuals: three carrying c.367del; p.Leu123* and one carrying c.1422del; p.Phe474Leufs*34 in *FGD1*. Additionally, we found one subject with a heterozygous missense variant in *PPP1CB* that causes Noonan syndrome-like disorder (MIM:617506) (c.146C>G; p.Pro49Arg). In conclusion, individuals in our cohort were found to carry pathogenic or likely pathogenic variants in genes associated with either RS or disorders with phenotypic overlap with RS. Our findings confirm that constitutive dysregulation of the noncanonical WNT pathway in humans gives rise to RS.

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Poster #46

Molecular and cellular mechanisms underlying SATB2-mediated craniofacial defects

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Human patients with mutations in the *SATB2* gene exhibit a variety of developmental defects, most of which are associated with deficiencies in neuronal and craniofacial development. Craniofacial defects characteristic of the *SATB2*-associated syndrome (SAS) include cleft or high-arched palate, micrognathia, ankyloglossia, and dental abnormalities. In addition to defects in the craniofacial skeleton, patients with *SATB2* mutations exhibit metabolic issues including abnormal thermal regulation, high metabolism, and sleeping disorders, suggesting a role for SATB2 in thyroid development. In this regard, *Satb2* is expressed in the neural crest mesenchyme of the pharyngeal arches and the foregut endoderm in the midline of the pharyngeal floor. Satb2 mutant mice have hypoplastic jaws and thyroid cartilage relative to wild-type. To further investigate molecular and cellular mechanisms of Satb2-mediated skeletal defects, we used the CRISPR/Cas9 system to generate Satb2 mutations in MC3T3-E1 cells. We found that mutations in *Satb2* decrease pre-osteoblast proliferation. Mutations in *Satb2* also cause chromatin defects including nuclear blebbing and donut-shaped nuclei. These defects may contribute to a slight increase in apoptosis in mutant cells, but apoptosis is insufficient to explain the proliferation defects. Instead, we hypothesize that Satb2 regulates genes associated with proliferation in pre-osteoblasts. Further testing of this hypothesis is ongoing in mouse and human cells.

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