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CONFERENCE REPORT

The Society for Craniofacial Genetics and Developmental Biology 47th Annual Meeting

Paul A. Trainor[1,2] | Timothy C. Cox[3] | David E. Clouthier[4] | Katherine A. Fantauzzo[4] | Matthew P. Harris[5] | Juhee Jeong[6] | Rolf W. Stottmann[7] | Amy E. Merrill[8]

[1]Stowers Institute for Medical Research, Kansas City, Missouri, USA | [2]Department of Cell Biology & Physiology, The University of Kansas School of Medicine, Kansas City, Missouri, USA | [3]Department of Oral and Craniofacial Sciences & Pediatrics, University of Missouri-Kansas City, Kansas City, Missouri, USA | [4]Department of Craniofacial Biology, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA | [5]Department of Genetics, Harvard Medical School; Department of Orthopaedics, Boston Children's Hospital, Boston, Massachusetts, USA | [6]Department of Molecular Pathobiology, New York University, College of Dentistry, New York, New York, USA | [7]Steve and Cindy Rasmussen Institute for Genomic Medicine, Wexner Research Institute, Nationwide Children's Hospital, Columbus, Ohio, 43205 and Department of Pediatrics, The Ohio State University School of Medicine, Columbus, Ohio, USA | [8]Department of Biomedical Sciences, Center for Craniofacial Molecular Biology, Herman Ostrow School of Dentistry, University of Southern California, California, Los Angeles, USA

Correspondence: Amy E. Merrill (amerrill@usc.edu)

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ABSTRACT

The Society for Craniofacial Genetics and Developmental Biology (SCGDB) hosted its 47th Annual Meeting on September 9–10, 2024, at the Stowers Institute for Medical Research and Children's Mercy Research Institute in Kansas City, Missouri. On the opening day, Drs. Jean-Pierre Saint-Jeannet and Elizabeth Leslie received the SCGDB Distinguished Scientist Awards in recognition of their exceptional contributions to craniofacial biology. Additionally, Dr. Daniel Jensen discussed his unique perspective on Treacher Collins syndrome, speaking as both a physician and a patient. Over the next 2 days, five sessions showcased groundbreaking research on cell signaling and genomic mechanisms regulating craniofacial development, human genetics, translational and regenerative approaches, and clinical management of craniofacial differences. The meeting also featured interactive workshops on engaging with journal editors during the manuscript review process and empowering mentees to take an active role in maximizing their mentorship experience. A poster session further fostered meaningful interactions among the attendees, who represented diverse career stages and research backgrounds in developmental biology and genetics, further strengthening the SCGDB community.

The 47th Annual Meeting of the Society for Craniofacial Genetics and Developmental Biology (SCGDB) was held on September 9–10, 2024, at the Stowers Institute for Medical Research and Children's Mercy Research Institute in Kansas City, Missouri. The local organizers were Drs. Paul Trainor, Timothy Cox, and Irfan Saadi. The meeting began at the National World War I Museum in Kansas City, where the SCGDB Distinguished Scientists Awards were presented to the 2024 honorees. This was followed by a keynote address from a pediatric otolaryngologist specializing in tracheostomy for children with craniofacial differences, and the evening concluded with a reception. The following 2 days of the meeting, which included workshops, four scientific sessions, a poster session, and a special clinical session on craniofacial differences, were convened in the Auditoriums

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at the Stowers Institute for Medical Research and Children's Mercy Research Institute. Below is a summary of the topics discussed, as well as the awards given.

1 | SCGDB Distinguished Scientist in Craniofacial Research Awards

1.1 | Chair: Dr. Amy Merrill, SCGDB President (University of Southern California)

The SCGDB presents annual awards, sponsored by Developmental Dynamics, to members for their contributions to the Society: The David Bixler Distinguished Scientist Award and the Marylou Buyse Distinguished Scientist Award. In the first session of the meeting, the awards were conferred to the 2024 honorees.

Dr. Jean-Pierre Saint-Jeannet, Professor at New York University (NYU) College of Dentistry, was the recipient of the 24 SCGDB David Bixler Distinguished Scientist Award in recognition of his long-term leadership and exceptional contributions to the field of craniofacial biology. Dr. Saint-Jeannet has pioneered the use of the *Xenopus* model system to model craniofacial syndromes and identify factors that regulate the development of neural crest cells (NCCs) and cranial placodes. This work has been published in more than 90 scientific articles and is supported by the National Institute of Dental and Craniofacial Research. Jean-Pierre has also made remarkable contributions to the field of craniofacial biology through his teaching, mentoring, and service. His excellence in teaching and mentoring has been recognized by a number of awards from NYU Dentistry. He is Editor-in-Chief of the scientific journal *Genesis*, serves on the Editorial Board of *Developmental Dynamics*, and was a standing member of a National Institutes of Health (NIH) review panel. From 2018 to 2022, Jean-Pierre served as the Treasurer Elect for SCGDB, where he played a key role in rebuilding the website, streamlining the financial workflows, and ensuring the success of the first virtual meeting during the COVID pandemic.

For over 25 years, Jean-Pierre's research has focused on mechanisms regulating craniofacial development, with a particular focus on using the *Xenopus* model to study the formation of cranial NCCs and placodes. NCCs, which arise from the dorsal part of the neural tube, are critical for forming the majority of skeletal elements in the face, whereas cranial placodes contribute to the sensory organs like the inner ear, olfactory epithelium, and optic lens. His interest in NCCs and cranial placodes began during his postdoctoral work at the NIH with Dr. Igor Dawid, where he employed the *Xenopus* model system to demonstrate the importance of WNT signaling in embryonic patterning. He found that WNT signaling provides essential cues for the formation of NCCs in the dorsal neural tube. In 1998, Jean-Pierre launched his independent research program as an Assistant Professor of Animal Biology at the University of Pennsylvania, where he was promoted to Associate Professor in 2004 and Professor in 2011. In 2012, he joined the faculty at NYU School of Dentistry and currently serves as Vice Chair for Research in the Department of Molecular Pathobiology. His independent research program has identified key gene regulatory networks controlling both NCC and cranial placode formation at the neural plate border.

Jean-Pierre's early independent work focused on the role of the SoxE family of transcription factors (Sox8, Sox9, Sox10) in *Xenopus* NCC specification. His lab demonstrated that Sox9 is required to initiate cranial NCC formation and otic placode development. They also found that Sox8 regulates NCC initiation, whereas Sox10 is essential for the formation of NCC-derived melanocytes. In later work, his lab built on these findings by identifying upstream regulators of genes that specify NCC fate through an analysis of the genes that control the specification of neural plate border cells. They discovered that modulating the expression of the transcription factors Zic1 and Pax3, which were previously implicated in NCC specification, shifts the fate of neural ectodermal cells from NCC to cranial placode cells in animal cap assays. Zic1 alone promotes cranial placode fate, whereas co-expression of Zic1 and Pax3 promotes NCC fate. They went on to show that although Zic1 is necessary and sufficient for induction of the cranial placode program, its expression does not overlap with placodal markers, indicating that Zic1-mediated regulation of placodal fate is nonautonomous. A microarray screen to identify Zic1 targets in the presence and absence of Pax3 revealed that Zic1 alone upregulates transcription of the retinoic acid (RA) transporter *Lpgds* and the RA-synthesizing enzyme *Raldh2*, creating an anterior source of RA that activates cranial placode specification in neighboring cells. Subsequent studies showed that Zic1-regulated RA acts on the preplacode region, activating expression of the transcription factor *Pitx2c* and the RA-degradation enzyme *Cyp26c*. This has led to a new model of cranial placode induction, where Zic1 both activates and contains RA signaling to drive placode differentiation, offering new insights into the precise control of cranial placode specification.

Jean-Pierre then described recent work on the developmental role of natriuretic peptide signaling during NCC and cranial placode formation. The microarray screen also revealed that co-expression of Zic1 and Pax3, which promotes NCC fate, upregulates transcription of Natriuretic peptide receptor 3 (*Npr3*) in the preplacode region and NCCs. They showed that the loss of *Npr3* or the natriuretic peptides *Anp* and *Cnp* disrupts both NCC and cranial placode formation. Npr3 functions as both a ligand clearance receptor and a signaling receptor that inhibits adenylyl cyclase to modulate cAMP levels. To explore which aspect of this activity is required for NCC and placode formation, they expressed a truncated version of human NPR3, lacking the intracellular signaling domain, and found that it rescued NCC formation but not cranial placode formation in Npr3-depleted embryos. This suggests that Npr3 clearance activity is critical for NCC formation, whereas its signaling activity is important for cranial placode formation. To further test this, they used an adenylyl cyclase inhibitor to successfully restore cranial placode gene expression in *Npr3*-depleted embryos. These findings establish natriuretic peptide signaling as a novel regulator of NCC and cranial placode fates.

Dr. Elizabeth Leslie, Associate Professor in the Department of Human Genetics at Emory University School of Medicine, was the recipient of the 2024 SCGDB Marylou Buyse Distinguished Scientist Award. This award, named after the first female president of the SCGDB, was created to recognize mid-career stage society members who have made important contributions to craniofacial biology. Elizabeth's research has revealed new

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insights into the genetics underlying phenotypic variation of orofacial clefts (OFC) and identified rare and common variants contributing to these conditions. This work has been published in high-impact journals and led to robust funding from the NIH. Elizabeth has also made an impact on the field of craniofacial biology as a member of the FaceBase Scientific Advisory Panel, a member of the ClinGen Craniofacial Malformations Gene Curation Expert Panel, and as Associate Editor of Human Genetics and Genomics Advances. In addition to her research, Elizabeth has served as a mentor to dozens of trainees, many of whom have received prestigious fellowships.

During her postdoctoral fellowship with Dr. Mary Marazita, Elizabeth used genome-wide association studies (GWAS) to identify common variants associated with OFC, including the first successful GWAS of cleft palate (CP). GWAS studies have identified over 50 loci linked to cleft lip (CL) and cleft lip and palate (CL/P), but fewer than 10 for CP alone, indicating distinct genetic mechanisms. In her independent research program, Elizabeth has focused on mapping the relationship between single nucleotide polymorphisms (SNPs) and specific OFC subtypes, a project she calls the "Cleft Map." Her lab's findings indicate that although CL and CL/P share some genetic associations, CL, CL/P, and CP each have distinct genetic risk factors. Additionally, some OFC SNPs exhibit opposite effects: A single SNP can increase the risk for CL while also being protective against CP, and vice versa. Genetic modifiers also play a crucial role in determining the severity and laterality of clefts. Her lab has shown that bilateral and unilateral clefts are influenced by different genetic modifiers, with some SNPs specifically increasing the likelihood of bilateral clefting without being associated with unilateral cases. Interestingly, these laterality modifiers were not previously reported in studies of combined CL/P, suggesting that subtype-specific analyses can unmask genetic associations not detected in previous analyses. She also described how a significant modifier locus for bilateral clefting was identified near *PAX1*, a gene associated with normal craniofacial phenotypes of the upper lip and nose.

CP can involve the hard and/or soft palate, and Elizabeth described her lab's GWAS analyses to identify subtype-specific genetic factors. This work identified a genome-wide significant locus for hard palate involvement near *ANGPTL2*, a gene expressed in the developing palate and limb, which interacts with RUNX2, a key regulator of bone formation. Notably, copy number variants in *ANGPTL2* are associated with both palatal defects and limb abnormalities.

Elizabeth's research also has clinical implications, particularly in genetic testing. Currently, most children with OFCs do not receive genetic testing, as isolated clefts with no family history are assumed to have a low recurrence risk (3%–5%). However, Elizabeth's lab has challenged this assumption by simulating clinical genetic testing on whole genome sequencing data from OFC parent-offspring trios from the NIH Gabriella Miller Kids First Pediatric Research Consortium. They found that approximately 10% of children with clefts carried a pathogenic variant, primarily in genes associated with dominant clefting syndromes. Notably, 20% of children with CP had a pathogenic variant, whereas the percentage among CL cases was much lower and indistinguishable from controls. These findings suggest genetic testing panels may be biased toward CP genes, raising questions about whether CL variants are harder to recognize. This work has also revealed some information about sex-specific differences in cleft prevalence, where CP occurs twice as frequently in females as in males, and CL/P occurs twice as frequently in males as in females. They found that the less commonly affected sex consistently had a higher rate of pathogenic variants, suggesting a higher concentration of disease-causing alleles or alleles with a stronger effect is required for disease presentation. This approach has also revealed the role of incomplete penetrance in OFC. Among multiplex families, 64% of transmitted variants came from affected parents. However, when considering all transmitting parents, penetrance dropped to 25%, indicating that some rare variants in well-established cleft genes can be present in unaffected individuals without negating their causal role.

Elizabeth then described her efforts to identify novel gene associations in unsolved cases of Van der Woude syndrome (VWS), an autosomal dominant clefting disorder affecting approximately 1 in 35,000 individuals. Work in the lab of Elizabeth's postdoctoral mentor Dr. Jeff Murray identified pathogenic variants in *IRF6* and *GRHL3* that cause VWS; however, about 20% of cases lack mutations in these genes. Using whole genome sequencing, Elizabeth's lab has identified *PRKCI* as a candidate gene for VWS. Although some individuals with *PRKCI* variants have classic VWS features, others exhibit additional traits such as developmental delay and autism, expanding the known clinical spectrum of the disorder. These findings improve the ability to diagnose and provide genetic counseling for VWS.

2 | Keynote Address

2.1 | Chair: Dr. Paul Trainor (Stowers Institute for Medical Research)

Bringing the award session to a close, **Dr. Daniel Jensen**, Assistant Professor of Surgery, and Medical Director of the Tracheostomy Program at Children's Mercy Hospital, Kansas City, gave the Keynote Address, titled "Zooming Out and Zooming In: Treacher Collins syndrome as a physician and patient." Dr. Jensen specializes in Otolaryngology–Head & Neck Surgery and Pediatric Otolaryngology and has retrospectively analyzed the efficacy of multidisciplinary craniofacial clinics, studied pain management and the effect of wound infections following tracheostomy, and researched the etiology of chromosome microdeletions in congenital disorders. Dr. Jensen is one of those rare individuals who is a patient, a patient advocate, a physician and a surgeon, and he provided his unique perspective on rare craniofacial conditions and how he researches and treats complex airway problems. Dr. Jensen also shared his personal experience of Treacher Collins syndrome. Using the analogy of being a hiker on a mountain trail, he described his life's journey, traversing the trail, with all its ups and downs, and his encounters with fellow hikers, such as his family, friends, physicians, surgeons, clinical geneticists and researchers that collectively have helped him to meet each challenge and reach the top. Dr. Jensen's presentation was an inspirational reminder of the importance of fundamental basic research and its clinical significance and impact.

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3 | Professional Development Workshops

Drs. Paul Trainor (Stowers Institute for Medical Research) and **Jean-Pierre Saint-Jeannet** (New York University) presented a workshop on how to influence editors and win over reviewers from their perspective as the current Editor-in-Chief of *Developmental Dynamics* and *Genesis*: *The Journal of Genetics and Development*, respectively. Key messages focused on selecting the appropriate journal, what editors are looking for when evaluating a manuscript, as well as a high-level summary of the peer review process. Journal selection can be driven by author's goals for speed and impact, the desired audience, and where similar papers are being published. A presubmission inquiry may be a useful tool and is offered by many journals. The aim and scope of a given journal is usually available on the appropriate website. Most journals also offer several types of articles beyond the typical research article. The editors stressed that initial efforts to prepare the manuscript with attention to detail and including the specific components required by each journal are well worth the extra time required. Although journals differ, all editors are looking for a compelling story supported by strong data coming to a clear conclusion. The importance of the cover letter was emphasized, as this is the one of the first impressions the author team will make on an editor. The cover letter should include a summary of the findings, highlight their significance to the field, and the explain their relevance to the target journal. A summary of the peer review processes was then described, outlining the steps from author submission to the editorial assistant's check-in, followed by evaluation by the Editor-in-Chief and/or Associate Editor, and finally, review by peer reviewers. A reasonable time frame for the peer review process was noted to be four to 5 weeks. Real examples of many parts of the process were shown and discussed. Although peer review is effective, the numerous challenges to the system were summarized. A discussion of submission success rates and leading reasons for rejection was followed by options for authors after receiving reviews.

Drs. Katherine Fantauzzo (University of Colorado Anschutz Medical Campus) and **Jeffery Bush** (University of California, San Francisco) presented a workshop on "Mentoring Up" A brief introduction provided a definition of key terms, followed by two case reports for small group breakout discussions among the audience members. Each group then shared their key takeaways with the larger group. A definition of mentoring was given as a relationship between a mentor and mentee based on a mutual desire for development toward goals and objectives. Scientists at all career stages and in all types of careers can benefit from mentorship. Choosing a mentor should be an intentional choice, considering both the goals of the relationship and the personality styles involved. Once the relationship is established, the presenters covered some tools to maintain effective communication and the importance of aligning expectations (and keeping attentive to those expectations). The opportunity to explore implicit biases and, using a mentoring relationship, to interact with colleagues from a different backgrounds and career levels were highlighted. Two case studies were again used to facilitate a second round of small group discussions. The first highlighted themes of the need to establish clear goals and expectations for performance by a graduate student from a mentor and the resulting confusion about how to best address a perceived inability to satisfactorily make sustained progress on a project. The second focused on a scenario where a trainee and faculty mentor had not set guidelines on what work was independent and could be taken from the lab. A robust discussion was had about both topics and reinforced the need for continued attention to mentoring relationships of all kinds.

4 | Scientific Session I: Signaling in Craniofacial Development

4.1 | Chair: Dr. Katherine Fantauzzo, SCGDB Secretary (University of Colorado Anschutz Medical Campus)

The first scientific session focused on signaling in craniofacial development. **Dr. Ronald Parchem** (Baylor College of Medicine), who was the plenary speaker, discussed cooperative regulation of neural fate specification by the MIR302 family of microRNAs (miRNAs), which repress translation by mRNA degradation or translation inhibition. The MIR302 family consists of four members, *Mir302a*, *Mir302b*, *Mir302c*, *and Mir302d* that reside on a polycistronic transcript. Upon folding into hairpin structures, the primary transcripts are cleaved to form 5p and 3p arms, resulting in a total of eight mature miRNAs across the family. The MIR302 3p miRNAs share a common seed sequence and represent 98% of expression across the family in E8.5 mouse cranial tissue, whereas the 5p miRNAs have divergent seed sequences. Additionally, non-canonical MIR302 family members are expressed as 5′ isoMiRs and expand the repertoire of seed sequences. The loss of *Mir302* has been shown to cause embryonic lethality with fully penetrant cranial defects in mice. Introduction of a single copy of *Mir302a* or *Mir302b* rescued the embryonic lethality and open neural tube phenotypes of *Mir302*-null mice. However, these mice exhibited decreased postnatal survival and noncommunicating hydrocephalus. Furthermore, although $Mir302^{a/a}$ and $Mir302^{b/b}$ mouse models were used to demonstrate that genomic clustering of MIR302 is not required for proper processing and abundance of the miRNAs in E8.5 cranial tissue, it is required for proper generation of 5′ isoMiRs. To determine how MIR302 family members regulate mRNA during cranial development, mRNA sequencing and matched miRNA sequencing were performed between wild-type and *Mir302*-null E8.5 cranial regions. These analyses identified 268 upregulated genes in *Mir302*-null samples, with a significant proportion of these containing a MIR302 seed sequence in the 3′ UTR. However, introduction of a single copy of *Mir302a* or *Mir302b* rescued the expression of the majority of these transcripts to wild-type levels, indicating that these two miRNAs repress the same predicted targets and are thus functionally redundant despite differences in abundance. AGO2 chimeric enhanced UV-crosslinking and immunoprecipitation was then used in human embryonic stem cells to identify MIR302:mRNA interactions. This analysis revealed 517 transcripts, including shared and unique MIR302a and MIR302b targets. Examination of isoMiR interactions in this same dataset demonstrated that MIR302c-3p, for example, uses different seed sequences for distinct target interactions. Validating this finding in vivo, the loss of MIR302 family members was shown to result in the upregulation of genes

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containing canonical and offset seed sequences in E8.5 mouse cranial tissue. Finally, although MIR302a and MIR302b have overlapping roles in the cytoplasm, ATAC-seq of *Mir302*$^{\Delta/\Delta}$, *Mir302*,$^{a/\Delta}$ and *Mir302*$^{b/\Delta}$ mouse models demonstrated that these miRNAs have different roles in regulating chromatin accessibility in the nucleus.

Next, **Dr. Greg Holmes** (Icahn School of Medicine at Mount Sinai) presented work on suture-specific regulation of coronal and lambdoid suture patency by Hedgehog interacting protein (HHIP) and parathyroid hormone-like hormone (PTHLH) activity in mice. The bones of vertebrate skulls are separated by fibrous joints called sutures, which are critical sites of skull growth during craniofacial development. Sutures consist of osteogenic fronts containing osteoprogenitors and a suture mesenchyme composed of fibroblast-like cells. Although the frontal and parietal bones at the coronal suture overlap, the parietal and interparietal bones at the lambdoid suture exist in the same plane. Craniosynostosis is a common birth defect resulting from premature fusion of the sutures. Single-cell analysis of the E18.5 mouse coronal suture previously revealed an enrichment of *Hhip*, which encodes a Hedgehog (HH) signaling inhibitor, in the suture mesenchyme. The loss of *Hhip* resulted in depletion of the suture mesenchyme and osteoprogenitors of the coronal suture by E18.5, as well as fusion of the lambdoid suture. Relatedly, osteogenic HH signaling was increased in the *Hhip*-null E18.5 coronal and lambdoid suture mesenchyme. *Pthih*, which inhibits Indian Hedgehog (IHH) signaling, is also expressed in the E18.5 coronal and lambdoid sutures and was upregulated in the osteogenic fronts and suture mesenchyme of *Hhip*-null mice. *Hhip*$^{-/-}$; *Pthlh*$^{-/-}$ double-homozygous mutant mice exhibited fused coronal sutures by E18.5 and a restoration of *Ihh* expression in the osteogenic fronts. Taken together, these results indicate that feedback inhibition of IHH signaling by HHIP is necessary for both coronal and lambdoid suture formation, whereas the coronal suture phenotype is buffered by HH-dependent *Pthlh* expression and activity.

Dr. Elizabeth Baretto (University of Calgary) spoke about the epistatic relationship between fibroblast growth factors (FGFs) 8 and 17 in embryonic craniofacial development. FGF signaling regulates the growth and morphology of several tissues during mouse development, and FGF8 in particular has previously been shown to contribute to craniofacial development. To explore the role of FGF8 in this context, her laboratory has employed an allelic series of *Fgf8* mouse models encompassing wild-type, *Fgf8*^{Neo}, *Fgf8*^{fl}, and *Fgf8*$^{\Delta 2/3}$ alleles. Previous work using this allelic series revealed a nonlinear relationship between *Fgf8* gene dosage and phenotype expression, in which a small change in gene expression led to a wide array of phenotypes. The phenotypic variability was pronounced when *Fgf8* was expressed below 50% of wild-type levels, whereas higher *Fgf8* expression resulted in phenotypic buffering. In this context, *Fgf17* expression was correlated with expression levels of *Fgf8*. Dr. Baretto is in the process of pairing the *Fgf8* allelic series with a *Fgf17* null allele to determine whether co-modulation of *Fgf8* and *Fgf17* alters the genotype–phenotype map during craniofacial development. A plan was presented to analyze approximately 30 mouse embryos from across 20 genotypes using microCT scanning of E10.5 embryos together with qRT-PCR and automated landmarking. To date, embryos have been harvested across 5 of these genotypes. Of note, *c-Myc* expression was significantly reduced in the *Fgf8Neo* mild hypomorphic model, which may explain the growth phenotype of these embryos.

The session was concluded by **Stefani Gjorcheska**, a PhD student from Cincinnati Children's Hospital Medical Center, who presented her work on the requirement for SOX10 in the systemic initiation of bone mineralization in zebrafish. Heterozygous variants in human *SOX10* result in Waardenburg syndrome, which is characterized by pigmentation and innervation phenotypes. Similarly, mutant models of this transcription factor in both mice and zebrafish recapitulate the human disease, with missing pigment and peripheral glia, deficient enteric innervation, and inner ear malformations. Although these models do not exhibit defects in neural crest (NC)-derived skeletal structures, a recently published zebrafish *sox10* loss-of-function allele, *sox10*^{ci3020}, presented with a significant delay in the mineralization of NC- and mesoderm-derived bones. This phenotype was confirmed in a second loss-of-function allele, *sox10*^{m618}. Although differentiating osteoblasts do not express Sox10 and osteoblasts differentiated normally in *sox10* mutant zebrafish, the collagenous matrix laid down by mutant osteoblasts did not mineralize. In addition, *sox10* mutant zebrafish exhibited significantly lower whole-body calcium content. Interestingly, the mineralization defect was not rescued with additional ambient Ca^{2+}, indicating a defect in calcium absorption. Relatedly, *sox10* mutants had significantly fewer Trpv6-positive epithelial ionocytes that mediate Ca^{2+} uptake. Furthermore, the anti-hypercalcemic hormone stanniocalcin was significantly upregulated in *sox10* mutant zebrafish because of an increase in the number of stanniocalcin-positive cells in these fish. The genetic loss of *stc1a*, encoding stanniocalcin, in *sox10* mutants rescued the bone mineralization phenotype, led to an increase in the number of Trpv6-positive ionocytes, and recovered calcium levels. Stanniocalcin is secreted by intermediate mesoderm-derived corpuscles of stannius (CS), which are surrounded by *sox10*-positive lineage cells, and *sox10* mutant zebrafish exhibited increased CS volume. A model was presented in which the larger CS in *sox10* mutant zebrafish leads to excess stanniocalcin, which in turn inhibits Trpv6-positive ionocyte production, resulting in reduced calcium uptake and delayed bone mineralization.

5 | Scientific Session II: Genomics of Craniofacial Development

5.1 | Chair: Dr. David E. Clouthier (University of Colorado Anschutz Medical Campus)

The second session of the meeting, focusing on the genomics of craniofacial development, was led by plenary speaker **Dr. Axel Visel** (Lawrence Berkeley National Laboratory), who described his lab's efforts to find and functionally annotate enhancers that regulate facial morphogenesis. As many enhancers regulating facial shape are also involved in craniofacial syndromes, his lab's early work included a collaboration with Dr. Jeff Murray, who had identified a SNP in a noncoding region near *IRF6*, a gene strongly correlated with CL. Axel's group discovered that this SNP was located in an enhancer active in the epithelium of the nasal prominences and that it destroyed an AP2a binding site.

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His group then conducted a large genome-wide scan of craniofacial enhancers, leading to the creation of the VISTA Enhancer Browser, which catalogs mouse enhancers along with their functional validation in transgenic reporter mouse embryos. Axel's group, in collaboration with Dr. Benedikt Hallgrimsson, demonstrated that knocking out three enhancers with similar reporter expression patterns altered skull shape, with each enhancer causing distinct structural changes.

Axel next detailed efforts to identify enhancers active in human craniofacial development through a collaboration with Dr. Stephen Lisgo. By analyzing H3K27ac histone marks (indicative of active enhancers) and ATAC-seq data (which highlights open chromatin regions), they identified thousands of putative enhancers across the genome. Although many of these enhancers are not conserved in mice, analysis using the Human Phenotypes Associated with Closest Genes tool (rGREAT tool) suggests that these nonconserved enhancers likely regulate processes that do not occur in mice. When combined with data from Dr. Justin Cotney's lab, Axel found that ~50% of enhancers were only active for short periods of time. Axel further showed data from a collaboration with Dr. Azeez Butali, who has performed whole genome sequencing on 130 trios in Ghana and Nigeria, in which patients have CL/P, but the parents are unaffected. Over 26 million variants were detected, though only ~7000 were de novo, novel, or very rare. When considering the validated craniofacial enhancers that contained more than one variant or were near known craniofacial genes, the number dropped to 175. One example was an enhancer for *Bmp2* that contained two variants, one of which disrupted a DLX3 binding site.

Axel ended with a description of a new project examining craniofacial variation during evolution. When comparing dolphins to other species, they have found that enhancers regulating midfacial length had undergone significantly more substitutions than seen in other species. Testing orthologs from six different species in his transgenic mouse reporter assays, only the dolphin enhancer did not produce midfacial staining. Interestingly, knocking out the element in mice resulted in a shape change to the very end of the premaxilla. They are currently knocking in the dolphin enhancer into mice to determine if further changes in craniofacial structures occur.

The next speaker was **Dr. Pierre Le Pabic** (University of North Carolina, Wilmington), who presented his lab's work on understanding the genomic and developmental mechanisms that underlie morphological diversity of the skull. Examining two Cichlid species from Lake Malawi, *Copadichromis azureus* (CA) and *Dimidichromis compressiceps* (DC), he found that the quadrate and preopercular were 26% longer in DC than in CA adults. Quantitative trait locus (QTL) mapping from CA and DC intercrosses identified a locus on chromosome 5, to which the gene(s) controlling jaw thickness and retroarticular length also mapped. All peaks overlapped the retinoic acid receptor g b gene (*rargb*), an exciting finding as retinoic acid (RA) signaling is known to be involved in skeletal development in animals and humans. Although another lab's model had RA signaling controlling the transition of osteoblasts to osteocytes, Pierre's lab hypothesized that it actually controlled the maturation of proliferative osteoblasts to nonproliferative osteoblasts. Unfortunately, homozygous *rargb* mutant zebrafish generated in the lab did not survive past 4 days post fertilization (dpf). However, soaking fish in the RARG agonist Palovarotene resulted in smaller bones that were over-ossified. This suggests that lower levels of Rargb in DC prolong the proliferative phase, resulting in larger growth zones and thus larger bones. One of the outstanding questions remaining is how variants in the *rargb* gene affect Rargb function. The lab is now investigating both the functional significance of specific variants in the *rargb* gene in addition to asking why there is differential use of the two promoters in the *rargb* locus across both tissues and developmental time.

The third speaker of the session, **Dr. Wei Zhang** (University of Southern California), spoke about his work investigating the relationship between aberrant ribosome biogenesis and craniosynostosis. The loss of Gli1-positive mesenchymal stem cells (MSCs) in the suture mesenchyme leads to craniosynostosis, though the role of ribosome biogenesis in this process is unclear. As the loss of the *Snord118* gene (encoding SnoRNA U8) affected brain organoid development, he performed a tissue-specific knockout of *Snord118* in suture MSCs using the *Gli1-CreERT2* strain. Three months after tamoxifen injection, they found partial fusion of the sagittal sutures in *Snord*$^{fl/fl}$; *Gli1-CreERT2* mice, accompanied by decreased proliferation and increased cell death of the suture mesenchyme. Based on this and other data, it appears that enhanced MSC differentiation in the mutants is accompanied by a decrease in bone resorption and remodeling.

Next, the lab found using differentiated human iPSCs that the loss of *Snord118* primarily affected translation and downregulation of ribosome protein genes, with RNA-seq analysis revealing downregulation of complement pathway genes in mutant cells, including *C3aR1*. To examine whether there is a genetic interaction between the complement pathway and ribosome biogenesis, they examined the sutures in mice lacking only *Snord118* in the suture mesenchyme or also globally lacking the *C3aR1* gene. Although *C3aR1*$^{-/-}$ mice did not have a suture defect, the decrease in suture mesenchyme observed in *Snord118*$^{fl/+}$; *Gli1-Cre* mice was more significant in *Snord118*$^{fl/+}$; *Gli1-Cre*; *C3ar1*$^{-/-}$ mice. Using an ex vivo suture model, they found that C3aR1agonist agonist treatment of mutant sutures caused sutures to remain patent. Thus, complement pathway activation partially rescues suture growth, suggesting that disruption of ribosome biogenesis may result in craniosynostosis because of changes in MSC function resulting from decreased complement activity.

The final speaker of the session was **Yilun Huang**, a PhD student from Cincinnati Children's Hospital Medical Center, who spoke on modeling spliceosomopathies in mice. Spliceosome members are associated with human craniofacial differences, the most common of which is craniofacial microsomia (CFM), affecting a number of organs and structures, including many in the face. As heterozygous variants in the *SF3B2* gene are associated with CFM, the lab generated a *Sf3b2* conditional knockout mouse. These mice were then crossed into the *Sox2-Cre* strain to produce an epiblast-specific knockout. Through breeding the subsequent mice, heterozygous *Sf3b2* mice were generated. They observed that *Sf3b2*$^{+/-}$ mice were developmentally delayed with asymmetric changes in nasal cartilage development. Additional changes included the transformation of the seventh cervical vertebrae into a first thoracic vertebrae and the sixth

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lumbar vertebrae into the first sacral vertebrae. These changes were not always symmetric. These rib changes have also been seen in *Sf3b1*$^{+/-}$ and *Sf3b4*$^{+/-}$ mice. A high penetrance of microphthalmia (70%) was also noted in *Sf3b2*$^{+/-}$ mice. Defects were present in both the lens and retina, whereas colobomas were sometimes present. At E12.5, reduced or absent expression of the lens markers *Cryaa* (crystallin alpha A) and *Foxe3* was observed, with changes also noted in the optic cup and optic stalk. Since many of the structures altered in CFM and in *Sf3b2*$^{+/-}$ mice are NCC-derived, she then crossed *Sf3b2*$^{fl/+}$ mice with the *Wnt1-Cre* strain to inactivate *Sf3b2* in premigratory NCCs. Resulting *Sf3b2*$^{fl/+}$; *Wnt1-Cre* mice were normal and viable. These findings suggest that SF3B2 acts in tissues other than NCCs during craniofacial development.

6 | Scientific Session III: Human Genetics of Craniofacial Development

6.1 | Chair: Dr. Timothy Cox (University of Missouri—Kansas City)

The third scientific session of the day focused on the important role of human genetics in providing new avenues of research for understanding the mechanisms underlying craniofacial development and dysmorphology. The session started with a plenary talk by **Dr. Seth Weinberg** (University of Pittsburgh) where he presented the findings of a major recent collaborative study to identify common genetic variants associated with normal-range variation in cranial vault shape. The study leveraged the extensive imaging and genetic data from two major repositories: The Adolescent Brain Cognitive Development (ABCD) study (a 10-year longitudinal study of ~11,000 adolescents with MRI scans and genome-wide SNP data) and the UK Biobank (MRI scans and genome-wide SNP data on ~66,000 adults). After quality filtering, his group used data from nearly 7000 individuals from the ABCD study as the discovery cohort, deploying digital segmentation methods on the 3D imaging data to quantify vault morphology and define multivariate shape traits for GWAS. The GWAS identified 30 genome-wide significant signals, with 20 replicated in a subset of ~17,000 individuals from the UK Biobank. Considering known intrinsic processes and extrinsic factors affecting vault development, such as biomechanical forces, the timing of cranial suture fusions, and the impact of growth and signaling from adjacent organs and tissues such as the brain, it was reassuring that the identified loci were enriched for genes involved in skeletal/bone development, morphogenesis, cartilage development, and odontogenesis. As with most GWAS studies, variants were enriched in enhancers—in this case, enhancers active in cranial NCCs and embryonic craniofacial tissue. Seth showed that some variants were associated with multiple vault shape traits, whereas others were restricted in their impact and, in many cases, were near biologically plausible genes like *RUNX2* and *ALX1*. Furthermore, the gene expression patterns of several hits corresponded with the observed phenotypic effects on vault shape.

Seth then described a follow-up study using whole genome sequencing data from 234 case-parent trios with isolated craniosynostosis available through the Gabriella Miller Kids First Pediatric Research Consortium to address whether variants near known suture-expressed genes might contribute to the risk for developing these isolated craniosynostoses. Indeed, this appears to be the case, with putative regulatory SNPs near *BMP2*, *BBS9*, and *ZIC2* being significantly associated with isolated sagittal craniosynostosis. These studies, therefore, help identify likely risk factors that also contribute to the penetrance and expressivity of more significant vault malformations and may also be important for better understanding changes in vault morphology through human evolution. Seth rounded out his presentation by highlighting ongoing work, including the preliminary data from an extension of the vault shape GWAS on nearly 53,000 participants from the UK Biobank, which suggests common SNPs may account for as much as 10% of the phenotypic variation in vault shape. Additional ongoing work is aimed at investigating other variant classes, gene–gene interactions, and the role of these variants in modifying craniosynostosis severity.

The second speaker was **Dr. Shuo Wei** (University of Delaware), who presented a fascinating study investigating the role of diphthamide deficiency in NC development. Diphthamide is a unique, evolutionarily conserved histidine modification found on eukaryotic translation elongation factor 2 (eEF2), a component of the ribosomal translation machinery. This complex amino acid modification has previously been shown to help ensure reading frame fidelity during translation and, thus, is critical for translation elongation. Although the loss of the diphthamide modification has long been known to be linked to developmental defects, the underlying mechanisms have remained poorly understood.

Shuo first highlighted the craniofacial presentation in a patient with compound heterozygous mutations (a truncating variant and a rare missense variant in a fully conserved catalytic residue) in *DPH1*, a gene involved in diphthamide biosynthesis. He also showed that *Dph1* was highly expressed in cranial NCCs in *Xenopus* and mouse embryos. To explore the underlying mechanisms, his team utilized *Xenopus tropicalis* and mouse models harboring the patient-specific mutations. In both models, depletion of *Dph1* led to decreased cranial NC marker expression, reduced cell proliferation in the neuroepithelium, and craniofacial defects. These defects were rescued in *Xenopus* embryos by a mutant eEF2 version that mimicked diphthamide modification but not by wild-type eEF2, highlighting the essential role of this modification in NC development. An unexpected but significant finding was identifying an additional function for eEF2. Without the diphthamide modification, eEF2 was found to dissociate from the ribosome and translocate to the nucleus, where it bound p53. Shuo then showed that this interaction promotes the transcription of p21, a cell cycle inhibitor, thereby leading to cell cycle arrest. Further supporting the idea that the diphthamide modification acts as a switch between two separate roles for eEF2—one in translation and another in transcriptional regulation of p53—Shuo showed that the knockout of one p21 allele could rescue the craniofacial and growth defects observed in the mouse model. This study, therefore, provides critical new insights into the molecular mechanisms by which diphthamide deficiency disrupts NC development and shows a central role for this eEF2 modification in both translational elongation and control of the p53-p21 pathway. Perhaps more significantly, his team's data suggest that the latter role is more important during craniofacial development than its previously established role in ensuring the integrity of the translational reading frame. The

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intriguing findings also highlight potential therapeutic targets for the rare condition of diphthamide deficiency.

Dr. Priyanka Kumari (University of Washington) was the third speaker of the session. She presented a large collaborative study that focused on understanding the genetic basis for the high incidence and regional distribution of CP cases in Finland. The Finnish population is unique both in its notably higher incidence of non-syndromic CP than CL or CL/P and in that it originated from a small founding population, which offers incredible power for genetics studies. To this end, Priyanka presented GWAS findings that identified a noncoding variant (rs570516915) located in a known enhancer (MCS9.7) for the *IRF6* gene that was strongly associated with CP risk in this population. Strikingly, the risk allele frequency of this variant correlated with the regional prevalence of CP cases in Finland, suggesting a causal or, at minimum, a strong contributory effect. Prior work had already identified a separate regulatory risk variant in MCS9.7 associated with an elevated CL/P risk, highlighting that regulatory variants in the same enhancer can confer risk to distinct types of facial clefts. Priyanka then described functional studies of the Finnish variant, including transient luciferase reporter assays and genome-edited human induced pluripotent stem cell-derived oral epithelial cells, to examine the effect of the Finnish risk variant on enhancer activity and *IRF6* expression, respectively. She provided evidence that the risk allele of rs570516915 disrupted the binding of the IRF6 protein to its own enhancer. These exciting findings support the notion that the high incidence and regional distribution of CP in Finland can be explained by this common, but high-impact regulatory variant that impacts *IRF6* expression through the disruption of its transcriptional autoregulation. Her presentation was an excellent example of the functional validation of regulatory variants that are increasingly appreciated as significant contributors to the population-level genetic risk for many common diseases.

Thoa Trung, a PhD student from the Stowers Institute for Medical Research, rounded out the session, speaking on the characterization of putative splice-disrupting variants identified in patients diagnosed with rare disorders of brain and craniofacial development. The study was a collaboration between Paul Trainor's lab and Scott Younger's lab at Children's Mercy Research Institute (CMRI). Variants were identified as part of the large Genomic Answers for Kids (GA4K) program at CMRI. Thoa briefly described the powerful initial cell-based screen for splicing anomalies performed by the Younger lab and then the bioinformatic pipeline used to prioritize these variants for further functional studies. Of note, they found a high prevalence of seizures among undiagnosed rare disease patients and cataloged over 4500 intronic variants in 347 genes affecting RNA splicing. Although she outlined the functional testing that is being done in both labs: Zebrafish modeling (Trainor lab) and patient-derived organoid modeling (Younger lab), the remainder of her presentation focused on her zebrafish phenotypic pipeline. This pipeline included survival testing at different ages, marker analysis, bright field morphological assessment, locomotor assays (touch-evoked response), confocal imaging of brain and craniofacial shape and size, and cell-specific marker quantification. Taking the audience through the pipeline, Thoa presented results for two CRISPR F0 mutants (*Ankrd11* and *Arid2*) that she has completed, although she highlighted that work characterizing CRISPR-derived zebrafish mutants of *Arid1b*, *Dcc*, *Ep300*, *Gli3*, *Pbx1*, *Ppp3ca*, *Reln*, *Setbp1*, *Sf3b4*, *Tnrc6a*, and *Wdfy* was ongoing. Overall, the study highlighted both the power of such functional screens as well as the rapid insight gained from testing the effects of rare intronic variants in the higher throughput zebrafish and patient-derived organoid models. Such studies will continue to enhance our understanding of pediatric disease etiology and pathogenesis.

7 | Scientific Session IV: Translational and Regenerative Approaches in Craniofacial Biology

7.1 | Chair: Dr. Matthew Harris, SCGDB Vice President (Boston Children's Hospital and Harvard Medical School)

The final scientific research session on Translation and Regenerative Approaches was kicked off by a plenary presentation by **Dr. Matthew Greenblatt** (Cornell University) on his lab's work identifying cell-specific populations and interactions maintaining suture patency. Focusing on stem cells of the calvarium, they characterize stem cell populations marked by Cathepsin K (CTSK) as a stem cell population specific to intramembranous bones and predominant within the suture. CTSK+ cells primarily mark the periosteum and are functionally distinct from endosteal cell populations. Deletion of Twist in these populations through CTSK-Cre recombination shows suture fusion, suggesting that altered developmental function within these populations of cells is sufficient to cause fusion. However, an analysis of fusion sites in the *Twist1*$^{fl/fl}$ mice showed a decrease of CTSK+ cells, suggesting replenishment of cells within the suture space by another lineage. Coincident with fusion, their lab identified these new cells driving inappropriate endochondral ossification. Thus, the intra-membranous specific CTSK cell lineages are replaced by an endochondral competent cell population. Analysis of human sutures from craniosynostosis patients also displayed such ectopic endochondral differentiation, suggesting conservation of these mechanisms.

Capitalizing on these experimental separable populations, through differential expression analysis, their group identified discoidin domain receptor (DDR2) marking complementary populations to CTSK cells while maintaining other cell surface markers previously used to specify stem cell populations. Through serial transplantation into the mouse kidney capsule or intramuscularly, DDR2+ cells self-renew and generate all other DDR2-lineage cells. These cells were surprisingly competent to drive endochondral ossification, and this occurred without inducing marrow differentiation.

These experiments led to the question of whether CTSK+ cells normally suppress the function of DDR2 and thus endochondral differentiation and suture fusion potential. Through transplant experiments in the forming mouse calvaria in which suture tissue has been ablated, sorted CTSK+, DDR2, or a mixture thereof was implanted. Results show that CTSK+ cells act to suppress the activity of the DDR2 cell population in generating fusion. Lastly, through transcriptional profiling of the CTSK+ and DDR2+ populations, his lab was able to identify differentially

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expressed components of these two populations. Of note are cases in which ligand- eceptor pairs were differentially regulated. An example was the expression of the ligand insulin growth factor 1 (IGF1) specifically in CTSK+ cells, whereas its receptor IGFR was expressed in DDR2 populations. They further show that supplementation of IGF1 by injection reduced suture fusion in *Twist1*-deficient mouse mutants, whereas conditional ablation of its receptor, IGFR, in wild-type mice caused increased suture fusion. Many of the known genetic regulators associated with synostosis are differentially expressed in these two cell populations, outlining a potential cell lineage etiology for craniosynostosis. Future work centers on an additional stem cell population they identified that, like DDR2, is competent for endochondral development; however, it can support hematopoietic differentiation of marrow.

The session followed with a talk by **Dr. Samantha Brugmann** (Cincinnati Children's Hospital) on targeting Sufu-mediated Gli transcription to alleviate deficiencies of cilia-mediated signaling in development. Primary cilia are microtubule-based extensions essential for the transduction of developmental signaling pathways. Of note, the posttranslational modification of hedgehog pathway members leading to the alteration of transcriptional activation. About 30% of known ciliopathies present with a characteristic set of craniofacial phenotypes, including midfacial anomalies, micrognathia, CL/P, as well as craniosynostosis. In her lab, they use a *Wnt1-Cre* mediated deletion of *Kif3a*$^{fl/lf}$ that mirrors ciliopathic phenotypes seen in patients. Analysis of the phenotypic presentation showed a duality in which high-hedgehog signaling caused midfacial phenotypes, whereas low-hedgehog signaling drove micrognathic phenotypes. Thus, simply replenishing or reducing hedgehog signaling can both alleviate as well as exacerbate disease phenotypes. In efforts to define aspects of nuanced control to alter hedgehog disruption in cilia deficiencies, the lab identified a known mediator of transcriptional mediation of hedgehog signaling, *suppressor of fused* (*SuFu*) as an actionable regulator to target, in part as SuFu is found highly complexed to GLI2 and GLI3 transcription factors when cilia are lost. They expanded evidence that a conserved amino acid D159 of SuFu acts as a patch in a presumed binding "clamp" to GLI transcription factors. They then generated a specific Sufu D159R knock-in mutant and asked if this could rescue their *Kif3a*$^{fl/fl}$ ciliopathy model. Unlike previous strategies, heterozygous D159R mice show balanced rescue of midfacial and mandibular phenotypes. Through DNA binding experiments, the lab showed that the addition of the D159R allele partially restored GLI3 binding to DNA, suggesting the rescue occurred through restoring transcriptional activity.

Dr. Katherine Woronowicz (Boston Children's Hospital and Harvard Medical School) the next session speaker, described a novel population of cells active in patterning the skull. Through comparative genomic analysis of selection associating with extended jaw proportions in the clade of beloniform fishes, a clade containing extreme examples such as halfbeaks and needlefish, Kate identified the *agap1* locus as a target underlying the evolution of the timing of extended jaw development. This locus was also found to be associated with human microsomia and reduced jaw size; thus, it was an interesting target for further exploration. Analysis of conservation at this locus identified a cluster of highly conserved enhancers (UCNEs) within *agap1* that were retained in all jawed vertebrates but were absent in jawless ancestors. Although *agap1* may be affected, the neighboring gene, a conserved homeodomain-containing transcription factor *gbx2*, which is associated with the specification of the NC, raised the question of whether this gene was specifically the cause of selection at the locus. Kate made transgenic zebrafish containing these enhancers and showed that their expression mirrored that of Gbx2 endogenous expression in the development of the jaw and a subpopulation of NC lineage cells at the boundary of the mouth. Interestingly, when these cells were examined more closely, they formed a stacked cartilage-like rod, but while structurally similar to cartilage, they did not retain classic cartilage markers. The group profiled the transcriptional signature of these cells and showed that differential expression of extracellular genes may characterize their function. However, it is unknown what these cells contribute to, as they were previously unknown. In the clade of beloniformes, large bills of halfbeaks were lost in the evolution of flying fishes. Looking at the genomic sequence of flying fishes, a specific deletion of this ultraconserved enhancer was identified. Using her transgenic assay, Kate showed that this element is necessary for midline expression, suggesting it evolved to repress overgrowth. This deletion removed a putative Alx binding site, and within *alx1* zebrafish mutants, ectopic midline cartilages are observed. This novel cell population also varies across different species having varied jaw structures, suggesting they may play a role in both the diversification of craniofacial structures as well as in human dysmorphology.

Arshia Bhojwani, a PhD student from the University of Southern California, was the final speaker of the session discussing her work on LGRs and connective tissues in zebrafish and mouse craniofacial development. Focusing on the jaw, Arshia performed single-cell analysis at developmental stages E16.5 and 18.5 in the mouse and found a unique, undefined cluster of cells. These cells expressed *Lgr5* with other connective tissue transcription factors such as *Scleraxis*. Lgr5 is a well-known regulator of stem cell biology in epithelia, specifically the intestine; thus, these findings raised the question of its role in connective tissue formation and regeneration. Using spatial transcriptomic analysis, she placed Lgr5 in context with the developing jaw joint and other connective tissue markers. Genetic lineage tracing of Lgr5 cells from E14.5 allowed specific analysis of the contribution of these cells to developing structures of the ligaments and tendons but found them absent from the cartilages of the condyle, Meckel's, and angular. Next, she showed that the loss of *Lgr5* function leads to a smaller angular of the jaw, which associates with tendons directly, suggesting that the presence of these structures is necessary for normal development. Current work on the potential redundant action of Lgr5 and Lgr4 shows additive functions of these alleles. Extending this work into zebrafish allowed analysis of different paralogues, as zebrafish do not have *lgr5* but rather *lgr4* and *lgr6*. Expression analysis showed that *lgr4* mirrors connective expression as seen for Lgr5 in the mouse. Lineage tracing with an nEOS transgenic zebrafish line, allowing photoconversion during development over time, showed the contribution of lgr4 cells to the perichondrium and tendon but not the cartilage of the jaw. Future work using loss-of-function alleles with these labeling tools will allow detailed analysis of the function of these cells in jaw formation. In addition, since *Lgr5* has been shown to mediate WNT signaling,

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efforts are ongoing to assess the role of this signaling pathway in connective tissue formation.

8 | Special Session: Clinical Management of Craniofacial Differences

8.1 | Chair: Dr. Juhee Jeong, SCGDB Treasurer (New York University)

The theme of the last session of the meeting was clinical management of craniofacial differences. **Dr. Janice Lee** (National Institute of Dental and Craniofacial Research) was the plenary speaker, and she discussed paradigms in the management of dentofacial differences. The main goals of clinical care are to achieve esthetic facial form, normal function, and psychosocial well-being. Several case examples were used to explain an elaborate process of evaluation, treatment, and outcome assessment by a multidisciplinary team. In addition, Dr. Lee presented how a large dataset of 3D skeletal imaging and geometric morphometric analyses is used to predict craniofacial growth of a patient, thereby helping the design of an intervention plan.

The second speaker was **Dr. Emily Farrow** (Children's Mercy Research Institute), who discussed the contribution of genomics to craniofacial disease management, primarily in rare disease diagnostics. She also introduced Genomic Answers for Kids (GA4K), a pediatric sequencing data repository at Children's Mercy Research Institute. Current efforts by Dr. Farrow and colleagues use a new technology of long-read sequencing (HiFi) to increase diagnostic rates of not only monogenic disorders but also complex disorders involving chromosome structure and epigenetic changes.

The last presentation of the session was from **Annette Campbell** (Rare Voices) and **Courtney Berrios** (Rare Voices Advisory Group, Children's Mercy Kansas City). Rare Voices is a stakeholder group in rare disease research comprising patients, patient families, clinicians, and researchers. The speakers emphasized the importance of community engagement in developing research agendas. Rare Voices used a step-by-step process including training, listening sessions, developing research questions, and community feedback surveys to come up with research topics focused on ethical, legal, and social aspects of rare diseases.

9 | Poster Sessions

9.1 | Organizer: Dr. Matthew Harris, SCGDB Vice President (Boston Children's Hospital and Harvard Medical School)

The second day of the meeting concluded with a 3-h poster session, in which faculty, postdocs, and students presented their work.

10 | Award Winners

10.1 | Poster Presentation Awards Sponsored by the American Association for Anatomy (AAA)

Winners of the poster presentation awards, sponsored by AAA, were Drs. Lorena Maili (Stowers Institute) (1st), Mohamed Hassan (Washington University) (2nd), and Kelsey Robinson (Emory University) (3rd) in the postdoctoral fellow category; and Emily Adelizzi (University of Iowa) (1st), Audrey Nickle (University of Southern California) (2nd), and Emmanuel Aladenika (University of Iowa) (3rd) in the graduate student trainee category.

10.2 | Platform Presentation Awards Sponsored by SCGDB

Winners of the platform presentation awards sponsored by SCGDB, which recognized impactful studies in craniofacial research, were Dr. Katherine Woronowicz (Boston Children's Hospital and Harvard Medical School) in the postdoctoral fellow category and Arshia Bhojwani (University of Southern California) in the graduate student trainee category.

11 | Summary

In conclusion, the 47th annual meeting of SCGDB brought together over 116 scientists to deepen our understanding of craniofacial development and disease. The event remains a premier platform for researchers of all career stages, genders, and backgrounds. We look forward to gathering again for the 48th annual meeting in Minneapolis, MN, in 2025.

Author Contributions

All authors contributed to both the writing and editing of this manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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