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CONFERENCE REPORT

The Society for Craniofacial Genetics and Developmental Biology 42nd Annual Meeting

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Abstract

The Society for Craniofacial Genetics and Developmental Biology (SCGDB) 42nd Annual Meeting was held at the MD Anderson Cancer Center in Houston, Texas from October 14–15, 2019. The SCGDB meeting included scientific sessions on the molecular regulation of craniofacial development, cell biology of craniofacial development, signaling during craniofacial development, translational craniofacial biology, and for the first time, a career development workshop. Over a one hundred attendees from 21 states, and representing over 50 different scientific institutions, participated. The diverse group of scientists included cell and developmental biologists and clinical geneticists, promoting excellent discussions about molecular pathways guiding abnormal cell behaviors and the resultant morphological changes to craniofacial development. The results were high-quality science and a welcoming environment for trainees interested in craniofacial biology.

1 | CAREER DEVELOPMENT WORKSHOP

The 42nd annual SCGDB meeting began with a career development workshop that included interactive discussions with the audience on "What to look for in a mentor?" led by Timothy Cox (University of Missouri-Kansas City) and Jean-Pierre Saint-Jeannet (New York University). Trainees were encouraged to develop and continually update an elevator speech about their research, as well as to consider mentors for different aspects of their career development. Importantly, discussions moved toward equality, equity and justice in science and how to pay it forward by being an advocate for others. The next session was "How do I deal with a manuscript rejection?" led by Editor-In-Chiefs from *genesis*, *The Journal of Genetics and Development* (Sally Moody, George Washington University) and *Developmental Dynamics* (Paul Trainor, Stowers Institute). The discussion included that publishing manuscripts has been a normal part of scientific communication for over 300 years (Royal Society of Edinburgh in 1731), and how to view rejection as a learning opportunity rather than a negative experience. There was also discussion on how to effectively peer-review our colleagues' work, and write constructive and empathetic reviews

(the way you would like your own work treated). The workshop concluded with an important discussion on "Harassment, unconscious bias and bullying—how do we deal with it?" led by Lisa Taneyhill (University of Maryland, College Park) and David Clouthier (University of Colorado Anschutz Medical Campus). This included a dialogue on changing perspectives and unconscious bias and harassment in science, distinguishing between explicit versus implicit bias, and how to effectively work with a power dynamic. Together, this session set the tone for the meeting as a welcoming atmosphere that encouraged trainees to be active participants in developing their career strategies.

2 | PLENARY SESSION I: SPECIAL AWARDS

2.1 | Chair: Sally Moody, SCGDB president (George Washington University)

For the first time, the SCGDB presented awards to members for their contributions to the Society: the Distinguished Service Award, the David Bixler Distinguished Scientist Award, and the Marylou Buyse Distinguished Scientist Award.

The SCGDB Distinguished Service Award was presented to **Geoffrey Sperber**, Professor Emeritus in the School of Dentistry of the University of Alberta. Professor Sperber is a long-term member of the Society and served as Secretary/Treasurer between 2003–2011. He is co-author, with Steven Sperber, of "Craniofacial Embryogenetics and Development", copies of which he signed at the meeting. Dr. Sperber has been previously distinguished by The University of Alberta by naming their Dentistry Museum, an annual lecture and an endowed research fund in his honor. Professor Sperber first provided a history of the Society, which was published in the program and is available on the SCGDB website (scgdb.org). He then described several aspects of craniofacial development and anomalies that are presented in his book, and explained their categorizations as "Malformations," "Deformations," "Disruptions," or "Dysplasias," giving several examples of each type.

The David Bixler Distinguished Scientist Award was created by the SCGDB and sponsored by *Developmental Dynamics* to honor the achievements of the Society's first President, Dr. David Bixler, a pioneer in salivary gland function and dental caries research. This award recognizes a senior scientist who has provided long-term distinguished leadership to the SCGDB and has made meritorious contributions to the craniofacial sciences. The first recipient of this award was **Joan Richtsmeier**, Distinguished Professor of Anthropology at Pennsylvania State University. Dr. Richtsmeier, a long-time member of the SCGDB, was recognized for her leadership in the Society, serving terms on the Executive Board as Vice President, President and Past President, as well as her many contributions to understanding craniofacial growth and evolution. The award was particularly meaningful to Dr. Richtsmeier as Dr. Bixler was honored at the first SCGDB meeting she attended. Dr. Richtsmeier explained how she became interested in craniofacial biology and the twists and turns and personal experiences that led to her long-term interest in osteogenesis and craniosynostosis. She ended by reminding the audience that what we are studying in our various animal models is "someone else's heartache"—in other words, the patients and their families that are dealing with craniofacial dysmorphologies should be on our mind as we explore their genetic and cell biological underpinnings.

The Marylou Buyse Distinguished Scientist Award was created by the SCGDB and sponsored by *Developmental Dynamics* to honor the memory of the Society's first female President, Dr. Marylou Buyse, a prominent clinical geneticist. This award recognizes a mid-career scientist who has made important contributions to the craniofacial sciences. The first recipient of this award was **Amy Merrill-Brugger**, Associate Professor in the Center for Craniofacial Molecular Biology at the University of Southern California. Dr. Merrill-Brugger reminded us that there are over 450 clinically recognized congenital disorders of the cranial skeleton. Dr. Merrill-Brugger explained how she started with a human disease to gain insight into how skeletal progenitor cells can become cartilage, bone, tendons and ligaments. Starting with human genetics, her postdoctoral work demonstrated that Bent Bone Dysplasia Syndrome (BBDS) can be caused by mutations in the FGF receptor 2 (Fgfr2) gene, which leads to the protein being mis-localized to the nucleolus—the site of ribosome biogenesis - instead of the cell membrane. Her cell biological studies demonstrated that Fgfr2 enhances the number of ribosomes produced, and BBDS mutations alter the stoichiometric ratios of 40S to 60S subunits. This mis-localization apparently prevents differentiation and maintains cells as bone progenitor cells. Her lab is pursuing studies to understand the molecular mechanisms that lead to the craniofacial defects in conditional *Fgfr2* knock-out mice.

The SCGDB is excited to partner with *Developmental Dynamics* to honor the exceptional contributions of our members.

3 | PLENARY SESSION II: MOLECULAR REGULATION OF CRANIOFACIAL DEVELOPMENT

3.1 | Chair: David Clouthier (University of Colorado Anschutz Medical Campus)

This session focused on genes and signaling pathways that regulate the development and differentiation of neural crest cells into the craniofacial complex. The session began with a plenary talk from **Julia Boughner** (University of Saskatchewan), who presented her work examining the integration of tooth and mandible development. While many of the same genes regulate the development of both tissues, p63 is required only for tooth development. Although the mandibles of *p63-null* mouse embryos look normal, morphometric analysis indicates that their growth becomes delayed sometime after E14. Gene expression analysis using microarrays and in situ hybridization revealed novel gene expression patterns downstream of p63, including *Cbln1*, *Pltp* and *Prss8*. The expression of these genes is conserved in gar fish and *Xenopus*, suggesting that a p63-driven network required for tooth development is evolutionarily conserved.

Rolf Stottmann (Cincinnati Children's Hospital Medical Center) next discussed his findings on the role of the primary cilia gene *Ttc21b* in facial development. *Ttc21b* mutant mice have craniofacial defects and microcephaly. Using a QTL approach in the *Ttc21b* background, *Gpr63* (on distal chromosome 4) showed a strong correlation with forebrain size. As a G protein-coupled receptor, Gpr63 exhibits brain-specific expression by E9.5, with analysis of *Gpr63* variants indicating that polymorphisms in *Gpr63* alter the frequency of localization to the primary cilia. While targeted inactivation of *Gpr63* did not result in a brain phenotype, double mutant *Gpr63;Ttc21b* mice have spina bifida, suggesting novel genetic interactions. Additional investigations have uncovered an association of *Ttc21b* with four genes identified in human genetics studies. Double homozygous mice (with $Ttc21b^{-/-}$) have more severe craniofacial phenotypes than do single homozygous mice, indicating new interactions in ciliogenesis and craniofacial development.

Rachel Keuls (Baylor College of Medicine) followed with a talk addressing how neural crest cells maintain developmental potential. Using small RNA sequencing, she identified a group of upregulated micro RNAs (miRNAs) that counterpoise the transition of pluripotent ectoderm to migrating neural crest cells. Among these, miR-302 was found to be required for the timing of neural crest cell migration in part by regulating *Sox9* expression. Deletion of *mir302* resulted in upregulated *Pax7* expression and precocious neural crest cell development, including premature delamination, with $Sox9^{+/-}$;$mir302^{-/-}$ mutant embryos showing a partial rescue of the precocious development phenotype. In contrast, overexpression of *mir302* reduced neural crest cell number. Based on additional sequencing and bioinformatic analyses, miR-302 appears to target multiple genes that establish a regulatory network required to balance neural crest cell formation and differentiation.

Andre Tavares (George Washington University) next described the identification of novel co-factors of Six1. Mutations in the *SIX1* gene and the gene encoding the SIX1 cofactor, EYA1, are present in half of Branchiootorenal (BOR) syndrome patients, leaving the identity of genes responsible for the remainder of cases unknown. Dr. Tavares showed that three potential co-factors identified by a screen in *Drosophila* (Sobp, Zmym2 and Zmym4) were expressed in the early *Xenopus* craniofacial region and acted either as transcriptional activators or repressors of Six1/Eya1 transcriptional activity. He also showed using either morpholinos or CRISPR/Cas9 knockdown in *Xenopus* that Sobp functions in neural border zone development and neural crest/placode formation, whereas Zmym2 and Zmym4 are required for neuroectoderm/ectoderm formation. These findings highlight other genes to examine in individuals with BOR syndrome.

Lindsey Barske (Cincinnati Children's Hospital Medical Center) concluded the session by describing the molecular networks directing gill cover morphogenesis. Gill covers are pharyngeal arch-derived appendages that develop in jawed fish, though their structure differs between bony and cartilaginous fish. Dr. Barske showed that the expression of the transcription factors *pou3f3a/b* directly correlated with these structural differences. In addition, ATAC-seq identified a *pou3f3* pharyngeal arch enhancer in jawed fish that is absent from lamprey, with sequence differences in this enhancer accounting for changes in gill cover structure between bony and cartilaginous fish. In *pou3f3a*/b mutants, posterior-directed growth of the gill cover commences but then collapses after five days, whereas overexpression of *pou3f3b* results in the formation of ectopic gill covers. These studies illustrate the importance of regulatory control of transcription factor expression during the development of the facial skeleton.

4 | PLENARY SESSION III: CELL BIOLOGY OF CRANIOFACIAL DEVELOPMENT

4.1 | Chair: Lisa Taneyhill (University of Maryland College Park)

In this session, researchers shared new findings on the cell biology of craniofacial development through the use of multiple animal models. **Amanda Dickinson** (Virginia Commonwealth University) anchored the session by discussing her work in *Xenopus* aimed at dissecting the molecular and cellular mechanisms underlying orofacial clefts. Her group performed RNA-seq on microdissected frog faces in the presence or absence of Retinoic Acid Receptor (RAR) in the neural crest and found many genes associated with human midfacial defects as well as epigenetic regulators such as *CHD1*, for which rare copy number variants exist in humans and are associated with cleft palate. Additional studies revealed craniofacial defects after CHD1 depletion via CRISPR or morpholinos, likely due to the increased apoptosis observed in migratory neural crest cells. Since the frog provides an excellent model in which to screen supplements that can prevent or reduce craniofacial defects, Dickinson showed that supplementation with folic acid could mitigate the increased apoptosis of the neural crest typically seen with CHD1 deficiency as well as in the presence of an RAR inhibitor. Next, Dickinson revealed new results from her lab addressing the role of vaping or e-cigarettes on the onset of craniofacial defects, particularly the potential toxicity of flavoring additives, which are inhaled instead of being metabolized via ingestion. Dickinson's lab once again exploited the advantages of the frog, focusing on the ciliated skin of the larva as a model of the human airway epithelium, to understand how these additives affect facial development. By exposing larvae to different aerosolized vaping additives, Dickinson demonstrated that many of these “flavorings” dramatically affect craniofacial development. One additive, Revel, caused several craniofacial defects in both the frog and mouse, including a narrow midface, eye abnormalities, smaller mouth, and clefting. Moreover, many of the defects are consistent with those seen upon inhibition of RAR signaling. To address this, the Dickinson lab performed RNA-seq on microdissected frog faces after exposure to Revel, which uncovered a subset of genes associated with human orofacial defects, including some of the same genes observed in their first data set. Collectively, these results suggest that Revel exposure alters RA signaling, leading to orofacial clefts, and further underscores the important of gene–environment interactions in human development.

Next, **Annita Achilleos** (Baylor College of Medicine), presented data regarding the role of Ronin, a zinc finger transcription factor that requires HCFC1 to transactivate its targets, in the Vitamin B12 pathway. One direct target of Ronin is *Mmachc*, which is required to convert inactive Vitamin B12 into its active form and causes Vitamin B12 (cobalamin) deficiency disorder type C (CblC) when mutated, a multisystemic disease whose pathophysiology is not known. Intriguingly, mutations in *HCFC1*, but not *MMACHC*, give rise to an X-linked variant of CblC called CblX, suggesting that these genes may function in a common pathway. Notably, mouse models with mutations in either *Ronin* or *Hcfc1* exhibit the stereotypical craniofacial anomalies observed in humans. To identify other genes besides *Mmachc* implicated in this pathway, Achilleos performed RNA-seq and ChIP-seq on wildtype and *Ronin* mutants, revealing that many ribosomal genes are direct targets of Ronin. Moreover, translation is severely compromised in the *Ronin* knockout mouse as indicated by polysome profiling. Taken together, these results demonstrate that CblX is both a cobalamin disorder and ribosomopathy, with mutations in *Ronin* and *Hcfc1* causing craniofacial defects through reduced transcription of *Mmachc* and ribosomal genes.

Katherine Fantauzzo (University of Colorado Anschutz Medical Campus) discussed new results from her laboratory regarding the role of the splicing factor Srsf3 in the palatal mesenchyme. Srsf3, an RNA binding protein that promotes splicing by facilitating exon identification, was identified as a target of PI3K/Akt-mediated PDGFRa signaling in a mass spectrometry-based phospho-proteomic screen. *Srsf3* is ubiquitously expressed but is enriched in the facial processes of mice during maxillary process fusion to generate the palatal shelves. Fantauzzo showed that Srsf3 is rapidly translocated to the nucleus upon its phosphorylation by Akt, where it can exert its effect on regulating alternative splicing. In support of this, RNA-seq analysis of palatal shelf mesenchyme derived from either wildtype or autophosphorylation mutant knock-in embryos in which PDGFRa is unable to bind PI3K, identified differentially alternatively-spliced transcripts containing Srsf3 binding sites that are associated with craniofacial defects. Finally, Fantauzzo demonstrated that conditional knockout of *Srsf3* in the neural crest leads to embryos with hypoplastic facial processes, midline facial clefting, and exencephaly, which may be due, in part, from the increased cell death in the facial mesenchyme. These data further underscore the importance of regulating proper RNA splicing during craniofacial development.

The roles of the matrix metalloproteinase MMP28 during craniofacial development were presented by **Nadege Gouignard** (New York University, Université Paul Sabatier), who uncovered *MMP28* expression in the placode cells in *Xenopus*. Interestingly, knockdown of MMP28 reduced the expression of various neural crest cell markers but only mildly affected placode cells, and these effects required the MMP28 catalytic domain. Suspecting that MMP28 would be secreted like other MMPs and affect neural crest cells non-cell autonomously, Gouignard generated a GFP-tagged MMP28, which was secreted but then accumulated in neural crest nuclei, as observed visually as well as after subcellular fractionation and immunoblotting. To further characterize MMP28 function, Gouignard used morpholino-mediated knockdown of MMP28 followed by expression of various mutant MMP28 constructs possessing changes in the nuclear export or nuclear localization sequences to dramatically shift the subcellular distribution of the protein. These experiments revealed a requirement for both nuclear localization as well as catalytic activity for rescue, suggesting that MMP28 is released from placode cells and works in a paracrine fashion through import into neural crest cell nuclei to regulate neural crest cell and later craniofacial development.

Junichi Iwata (University of Texas Health Science Center at Houston) discussed the role of cholesterol in the context of the primary cilium and craniofacial development. Using a mouse model of Smith-Lemli-Optiz Syndrome possessing a mutation in *7-dehydroxycholesterol reductase* (*Dhcr7*), which has high levels of cholesterol intermediates and low levels of mature cholesterol, Iwata showed that these mice have accelerated bone formation, leading to a lack of sutures, smaller bodies, suckling defects, and cleft palate. Moreover, these phenotypes are due, at least in part, to increased osteogenic differentiation. To address how aberrations in cholesterol metabolism lead to altered bone formation, Iwata examined the nonmotile cilium and found that *Dhcr7* knockout osteoblasts are compromised in their cilium formation, with many cells never forming cilia due to the accumulation of primary cilium vesicles and thus inability to properly make the cilium membrane. Upon further investigation, Iwata found that *Dhcr7* mutants have decreased Hedgehog signaling but increased canonical Wnt signaling, with the latter pathway being more dominant as evidenced by the rescue of the *Dhcr7* knockout phenotype upon loss of one allele of *Axin 2* to dampen Wnt signaling. Intriguingly, these bone phenotypes in the *Dhcr7* knockout mouse can also be rescued pharmacologically through administration of statins to normalize cholesterol levels. As such, proper regulation of cholesterol and its intermediates is critical for ciliogenesis, which ultimately impacts osteogenesis and skull formation in the developing embryo.

5 | PLENARY SESSION III: TRANSLATIONAL CRANIOFACIAL BIOLOGY

5.1 | Chair: Timothy cox (University of Missouri-Kansas City)

The Translational Craniofacial Biology session included talks on the publicly available resources to support disease model research through the identification of new genes and mechanisms contributing to common facial birth defects. The plenary presentation by **Steven Murray** (The Jackson Laboratory) highlighted the contributions the Jackson Laboratory has been making as part of the International Mouse Phenotyping Consortium (IMPC). Each node of the IMPC is funded to provide systematic functional annotation of the mouse genome with a particular focus on genes for which there are little or no experimental data currently available. Murray highlighted the standardization of functional data collection across the nodes, including staged embryo and adult phenotyping. This standardized phenotyping pipeline included viability assessments and a gamut of physical and biochemical measurements from all organ systems. He presented summary statistics on the more

than 8,500 individual gene knockouts (traditional and now CRISPR/Cas9-mediated) that have been produced so far and the future goals of the program. He addressed the consistent observation between nodes that around one third of all knockouts are embryonic lethal, with this list already being enriched for known human disease genes. He emphasized that all data are deposited and available to the public in a searchable online resource. Finally, Murray presented proof-of-principle studies from his group, demonstrating the feasibility of direct phenotypic analysis of F0 ("founder") mosaic-edited mouse embryos generated by CRISPR/Cas9, which have the potential to improve the speed and utility of the mouse as a tool to validate and model novel variants for human disease.

Soma Dash (Stowers Institute) presented her data on *Med23* as a new candidate gene for Pierre-Robin Syndrome (PRS). PRS is a craniofacial disorder characterized by micrognathia (small lower jaw) and glossoptosis (retracted tongue position) that can cause difficulty in swallowing and neonatal respiratory distress. Patients often have cleft palate that is thought to be secondary to a physical block of palatal shelf elevation and/or fusion by the abnormal tongue position. Dash described the generation of mice in which *Med23* was conditionally deleted in the cranial neural crest cell population, producing a phenotype similar to PRS. Notably, Dash showed that *Sox9*, the only currently known gene linked to PRS, was upregulated in the conditionally targeted embryos compared to controls, supporting a cell-autonomous requirement for *Med23* in cranial neural crest cells and potentially linking *Med23* to the pathogenesis of PRS.

Courtney Willett (Emory University) described studies on a multiplex family of Colombian ancestry in which five individuals from two generations presented with non-syndromic cleft lip with or without cleft palate (NS-CL/P). Exome sequencing did not reveal any segregating single nucleotide variants of note. Members of the family also had previously been included as part of a genome-wide association study, the data from which was re-utilized by Willett to instead search for chromosomal anomalies (insertion/deletions, or indels). Willett described a novel ~3 Mb deletion on 6p24 in all affected individuals. None of the 12 known genes found within the deleted region had functions or expression consistent with a major role in the phenotype. However, a known clefting gene, *TFAP2*, was located 3′ to the breakpoint. Mutations in *TFAP2* had previously been shown to cause Branchio-Oculo-Facial syndrome (BOFS), a dominant condition characterized by ocular anomalies including microphthalmia, a dysmorphic facial appearance including hypertelorism, a broad nasal tip, carp mouth and often cleft or "pseudo-cleft" lip/palate, branchial sinus defects, and anomalies of the external and middle ear. Re-evaluation of the family from photos and medical records revealed a variable facial appearance in affected individuals that included hypertelorism, malformed nasal tip, carp mouth and an ear pit in one person, although no individual met the criteria for a diagnosis of BOFS. Assessment of the genomic region revealed the 3′ breakpoint of the deletion to be located in the middle of a conserved 2.5 Mb topologically associated domain containing *TFAP2A*. In addition, the deletion was found to remove at least one characterized enhancer element that is active in human cranial neural crest cells and human fetal craniofacial tissue and a second with activity in neural crest cells in mouse embryos. The evidence presented by Willett concluded that the deletion has a positional effect on *TFAP2A* expression, resulting in mild features of BOFS, and that such mutational types may underlie other case of NS-CL/P.

Continuing on the theme of NS-CL/P, **Bhavna Tandon** (University of Texas Health Science Center at Houston) talked about her research on matrix metalloproteinase 2 (*mmp2*). *MMP2* became a gene of interest to their group based on RNAseq data that revealed the gene was differentially expressed between wildtype zebrafish and *crispld2* morphants. Variants in human *CRISPLD2* had previously been shown to be significantly associated with NS-CL/P susceptibility in multiple Caucasian and Hispanic cohorts. *crispld2* encodes a secreted protein and was previously suggested to have a role in regulating cranial neural crest migration. In support of a functional role for *mmp2* in cranial neural crest cells, Tandon reported that low concentration morpholino knockdown of zebrafish *mmp2* resulted in embryos with smaller heads and an abnormal mandibular arch skeleton. Furthermore, using their cohort of Hispanic families, she described the significant association of a variant, rs243836, in *MMP2* with human NS-CL/P. Tandon concluded that their data point to a *CRISPLD2*-linked role for MMP2 in the development of the craniofacial skeleton and suggested that their zebrafish approach provides a useful model to test putative genes associated with NS-CL/P in vivo.

Ela Knapik (Vanderbilt University) also described the use of zebrafish, this time to uncover the primary genetic cause of a craniofacial condition and provide insight into its pathomechanism. Knapik described using a phenotype-driven forward genetic screen to identify the *ric1* gene, encoding a component of a guanine-exchange factor (GEF) complex, as essential for normal craniofacial development. Investigating the basis for the abnormal craniofacial phenotype, Knapik's team showed that the Ric1 GEF complex activated Rab6a GTPase and that this activation was required for secretion of collagen into the extracellular matrix. In an elegant example of how model organism research can yield important translational insight, Knapik described using a gene-based phenome-wide association study (PheWAS) based on data available in a unique resource available at Vanderbilt: the EHR-linked BioVU biobank. This resource provide researchers access to deidentified electronic health records (EHR) of more than 2.8 million patients and around a quarter million DNA samples (BioVU) from a subset of those patients. Using this resource, Knapik's team showed that reduced expression of *RIC1* is associated with skeletal and dental conditions. Further, they found that re-evaluation of the clinical features of individuals homozygous-by-descent for a rare variant in *RIC1* revealed the patients to have many signs of the BioVU associated phenome, terming this novel Mendelian craniofacial syndrome CATIFA (Cleft lip, cAtaract, Tooth abnormality, Intellectual disability, Facial dysmorphism, ADHD). Consistent with the function demonstrated for Ric1, zebrafish cells and CATIFA patient skin fibroblasts were found to accumulate intracellular procollagen in the trans-Golgi network, and the identified human *RIC1* variant failed to rescue the procollagen secretory defects in zebrafish

mutants, thus, establishing pathogenicity of the variant and evolutionary conservation of this procollagen secretory pathway. These studies provide an example of the power of making accessible the vast resource of existing EHRs, especially when linked to biological samples. The talk was a wonderful way to end an exciting translational science session.

6 | PLENARY SESSION IV: SIGNALING DURING CRANIOFACIAL DEVELOPMENT

6.1 | Chair: Jean-Pierre Saint-Jeannet (New York University)

The last session of the meeting focused on the signaling pathways regulating craniofacial development. The plenary presentation by **Andrew Groves** (Baylor College of Medicine) described the work of his group characterizing the role of the forkhead box transcription factor, *Foxi3*, in mouse inner, middle, and external ear development. *Foxi3* is expressed in the pre-placodal ectoderm and is downregulated as otic induction proceeds. *Foxi3* mutant mouse embryos failed to form an otic placode, or express genes required for otic and inner ear development such as *Pax2* and *Sox9*. To understand the mechanisms by which Foxi3 induces otic placode formation, Groves used an ESC model of otic placode induction. RNA-seq analysis of gene expression in wildtype and *Foxi3* mutant ESCs, showed a surprising and consistent upregulation of *Hox* genes in mutant cells. This result points to a model in which Foxi3 acts positively to prepare otic genes for transcription, and negatively to repress neural and neural crest patterning genes. Interestingly, Foxi3 is not only required for inner ear formation, it is also necessary for middle and external ear development. *Foxi3* mutant mouse embryos have severe pharyngeal arch defects and lack middle ear ossicles. This phenotype was traced back to a loss of *FGF8* expression in the pharyngeal ectoderm, which is required for neural crest cell survival in arches 1 and 2. Finally, Groves discussed recent evidence linking Foxi3 variants/deletions to external ear defects (microtia) in humans and hairless dogs.

Jaeho Yoon (National Cancer Institute at Frederick) next described his findings that provide novel insights into the pathogenesis of DOORS (deafness, onychodystrophy, osteodystrophy, and mental retardation) syndrome, a condition characterized by mental retardation, sensorineural deafness, abnormal nails and phalanges, seizures and occasional craniofacial dysmorphology. In humans, DOORS has been linked to mutations in the *TBC1D24* gene, which encodes a Rab35-GTPase activating protein (Rab35 GAP). In *Xenopus* embryos, *TBC1d24* is expressed in migrating cranial neural crest cells where it interacts with the transmembrane ligand, ephrinB2. Interestingly, this interaction is mediated by the Wnt signaling scaffold protein Disheveled (Dsh), which targets *TBC1d24* to the plasma membrane. Knockdown of *TBC1d24* or ephrinB2 function in the embryo caused cranial neural crest cell migration defects, affecting primarily contact inhibition of locomotion (CIL). Biochemical and cell biological analyses indicate that disruption of the ephrinB2/Dsh/TBC1d24 complex upon phosphorylation of ephrinB2 by its Eph receptor, resulted in increased E-cadherin expression at the plasma membrane, which presumably is the cause for disrupted CIL and cranial neural crest cell migration defects. These results suggest that TBC1d24 is a novel regulator of ephrinB2-dependent neural crest cell migration through a mechanism that involves E-cadherin recycling in these cells.

Xiaolei Zhao (University of Texas McGovern Medical School) next described a novel role for Hippo signaling during neural crest formation and its interaction with the Wnt/β-catenin signaling pathway. Conditional inactivation of the Hippo effectors, Yap and Taz, in the mouse neural crest lineage caused cranial bone defects and failure of neural crest cells to proliferate, migrate and differentiate. The link to the Wnt/β-catenin pathway stemmed from ATAC-seq data from isolated mouse craniofacial tissues showing that a large number of genes in this analysis were co-enriched for Tead (a Yap/Taz cofactor) and Tcf/Lef (β-catenin cofactors) motifs, suggesting that these pathways co-regulate neural crest gene expression. This was confirmed for at least two Wnt targets critical for cranial neural crest specification, *Twist* and *Sox9*. Interestingly, co-immunoprecipitation analysis indicates that Yap/Taz and β-catenin physically interact in isolated neural crest cells. These results highlight the importance of Hippo signaling in neural crest development and its interplay with the Wnt/β-catenin pathway, a known regulator of both neural crest and craniofacial development.

For the last presentation of the meeting, **Ariadne Letra** (University of Texas Health Science Center at Houston) discussed efforts to elucidate the basis for the phenotypic and genotypic heterogeneity associated with tooth agenesis (TA), a common craniofacial condition characterized by failure of permanent tooth development. Because *WNT10A* has been proposed as a major gene linked to TA, 13 rare *WNT10A* variants, previously associated with TA, were analyzed for their activity after transfection in tooth-derived multipotent stem cells, known as SHED (stem cells from human exfoliated deciduous teeth). In a TopFlash assay that measures Wnt activity, 10 WNT10A variants exhibited low or no luciferase activity as compared to wild type, indicating that the mutant proteins were unable to activate WNT signaling. In addition, the mutant transfected cells presented decreased WNT10A protein expression, reduced binding to Frizzled receptors, and altered expression of several genes associated with tooth development: *PAX9*, *MSX1*, *AXIN2*, and *RUNX2*. These results suggest that *WNT10A* variants may have distinct biological activities, which may represent a contributing factor in the expressivity of the TA phenotypes.

7 | SUMMARY

In summary, the 42nd annual meeting of the Society for Craniofacial Genetics and Developmental Biology in Houston, Texas brought together a diverse group of scientists to present and discuss cutting edge approaches to better our understanding of craniofacial development and alterations that promote pathogenesis. The meeting

continues to be as an outstanding forum for the craniofacial sciences and a welcoming environment for all researchers regardless of the stage of their career, gender or background. We look forward to convening for the 43rd annual meeting of the Society for Craniofacial Genetics and Developmental Biology in Pittsburgh, Pennsylvania, October 19–21, 2021.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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