Received: 23 January 2021 | Accepted: 20 February 2021

DOI: 10.1002/ajmg.a.62150

CONFERENCE REPORT

AMERICAN JOURNAL OF medical genetics PART A WILEY

The society for craniofacial genetics and developmental biology 43rd annual meeting

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Funding information
American Association for Anatomy; Developmental Dynamics

Abstract

The Society for Craniofacial Genetics and Developmental Biology (SCGDB) held its 43rd annual meeting in a virtual format on October 19–20, 2020. The SCGDB meeting included the presentation of the SCGDB Distinguished Scientists in Craniofacial Research Awards to Marilyn Jones and Kerstin Ludwig and four scientific sessions on the molecular regulation of craniofacial development, craniofacial morphogenesis, translational craniofacial biology, and signaling during craniofacial development. The meeting also included workshops on career development, NIH/NIDCR funding, and the utility of the FaceBase database, as well as two poster sessions. Over 190 attendees from 21 states, representing over 50 different scientific institutions, participated. This diverse group of scientists included cell biologists, developmental biologists, and clinical geneticists. While in-person interactions were missed due to the virtual meeting format imposed by the COVID-19 pandemic, the meeting platform provided ample opportunities for participant interactions and discussions, thus strengthening the community.

KEYWORDS
cleft lip, cleft palate, craniofacial, genetics, neural crest

1 | CAREER DEVELOPMENT WORKSHOP

1.1 | Chair: Dr. Sally Moody, SCGDB president (George Washington University)

The 43rd annual SCGDB meeting began with a career development workshop led by Sharon Milgram, Director of the Office of Intramural Training and Education (OITE) at the National Institutes of Health. Dr. Milgram focused her talk on recognizing the stress of training during a global pandemic and dealing with its uncertainties in a productive manner. Her introductory slide captured this theme: "We are all forever changed by the experience we are going through now…let's make sure something positive comes of it." She presented data from the Wellcome Trust and several publications that demonstrate that the somewhat traditional research culture of unhealthy competition results in harassment and mental health issues, which then negatively impact research productivity and career advancement. To improve the research culture, Dr. Milgram advocated for the promotion of wellness strategies and collaborations between principal investigators, trainees, and institutional resource staff to help individuals develop the resilience needed to be successful in academic and research careers. She presented the NIH OITE Wellness Model and explained how its various aspects —wellness of body, mind, heart, and spirit—provide the foundation to resilience. Dr. Milgram also discussed the stigma often felt about choosing a career path outside of academia or research institutes, and how we all need to support diversity in the career outcomes of our trainees. Finally, she advocated for trainees to engage multiple mentors to address different career aspects and thoughtfully select a training environment that best matches one's personality and needs; she provided many resources for improving mentor–mentee relationships. The slides of Dr. Milgram's talk, which include links to several resources on these topics,

are posted on the SCGDB web site (https://www.scgdb.org/new-page-past-meet). This session set the tone for the meeting as a welcoming atmosphere for trainees to consider career pathways as well as to assess their strategies for resilience during the COVID-19 pandemic.

2 | NIH–NIDCR FUNDING

2.1 | Chair: Dr. David Clouthier, SCGDB vice president (University of Colorado Anschutz Medical Campus)

The NIH–NIDCR funding workshop, which covered aspects of NIDCR research project grants and awards to support mentored research training and career development in dental, oral, and craniofacial research, was presented by Drs. Leslie Frieden (Extramural Training Officer) and Katherine Stein (Program Officer and Director, Developmental Biology and Genetics Program). Their presentation provided an overview of the grant application process, training award mechanisms, and investigator-initiated grant mechanisms and concluded with a summary of several current programmatic interests within the NIDCR that relate to craniofacial development. Contact information for Drs. Frieden and Stein can be found at www.nidcr.nih.gov

3 | FACEBASE WORKSHOP

3.1 | Chair: Dr. Samantha Brugmann, SCGDB secretary (Cincinnati Children's Hospital Medical Center)

Mr. Rob Schuler from FaceBase presented the third workshop of the meeting. FaceBase is an open, comprehensive resource for dental and craniofacial research which fosters data sharing and curated resources relevant to the entire community (www.facebase.org). The platform itself is curated by a core team with craniofacial and computer science expertise to provide both an advisory role and oversight. With 942 datasets currently available, FaceBase continues to grow and evolve into a resource that is utilized by scientists across the globe. Mr. Schuler presented background information to explain the organization of the platform and walked attendees through how to search the platform to find data most relevant to their own work. He also shared that future outreach will be provided to encourage and assist members of the community with uploading their own data. The slides of Mr. Schuler's talk are posted on the SCGDB web site (https://www.scgdb.org/new-page-past-meet).

3.2 | Chair: Dr. Sally Moody, SCGDB president (George Washington University)

The SCGDB presented awards to members for their contributions to the Society: the David Bixler Distinguished Scientist Award and the Marylou Buyse Distinguished Scientist Award.

The David Bixler Distinguished Scientist Award is named in honor of the achievements of the Society's first President, Dr. David Bixler, a pioneer in genotyping craniofacial syndromes. This award recognizes a senior scientist who has provided long-term distinguished leadership to the SCGDB and has made meritorious contributions to the field of craniofacial sciences. The recipient of this year's award was Dr. Marilyn Jones, distinguished professor of Clinical Pediatrics at the University of California, San Diego (UCSD) and Director of the Cleft Palate and Craniofacial Treatment Programs at Rady Children's Hospital. Dr. Jones received her MD from Columbia University. She completed her residency at UCSD, during which time she served as Chief Resident in Pediatrics, followed by a fellowship in dysmorphology. Marilyn is an internationally renowned clinical geneticist and dysmorphologist who has had a profound impact on the field of craniofacial anomalies through her extensive publications, service to professional societies, and patient advocacy groups. She has also made significant contributions to the SCGDB by serving as an organizer of two very successful annual meetings.

Dr. Jones began her talk with a fascinating description of Dr. Bixler's work and the history of identifying the multifactorial inheritance of cleft lip with or without cleft palate (CL/P), based on the work of Poul Fogh-Andersen and Clark Fraser in the 1970s. Dr. Jones, as the head of Dysmorphology and Genetics, decided to build upon these studies by providing a more detailed description of clefting from her nearly 3000 patients to better understand the etiology of the dysmorphologies. She discussed the importance of analyzing multiple malformation syndromes of CL/P versus isolated cases. By using this approach, her group has been able to identify the etiology, including chromosomal abnormalities, microdeletions, single-gene mutations, and teratogens, in about 60% of her patients. Interestingly, the frequencies of patients in these categories differ between the groups with CL/P versus cleft palate only (CPO). For the family of the patients, having an etiological explanation helps tap into information for longitudinal prognosis and for recurrence risk counseling.

Dr. Jones finished her presentation by emphasizing that knowledge of underlying etiology for syndromes informs surgical and speech outcomes. She presented two examples of how knowledge of the genetic basis of a syndrome informed susceptibility to nonsyndromic clefting: *PVRL1* in Margarita Island Ectodermal Dysplasia and *IRF6* in nonsyndromic CL/P. Dr. Jones reiterated the importance of careful descriptions of craniofacial phenotypes into subtypes to better guide whole-genome assessments that tend to lump nonsyndromic clefting cases together. She concluded with a message for the junior members of the audience: "when someone opens the door and gives you a chance, you need to walk through it because you are not going to know what opportunities are on the other side."

The Marylou Buyse Distinguished Scientist Award was created by the SCGDB 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 recipient of this award was Dr. Kerstin Ludwig, the Emmy-Noether Junior Group leader in

Craniofacial Genomics in the Institute of Human Genetics at the University of Bonn, Germany. Dr. Ludwig received her Ph.D. in Molecular Biomedicine from the University of Bonn during which time she revealed important insights into key population-level susceptibility loci for CL/P. Since then, she has risen up the academic ranks at the University of Bonn to become Junior Group Leader and Head of the Next-Generation Sequencing Facility. Kerstin has published extensively on the complex genetics of nonsyndromic orofacial clefting and served on numerous editorial boards and review panels. In her talk on recent advances and future challenges of orofacial clefting genetics, Dr. Ludwig first discussed different ways of sorting patients into subgroups of orofacial clefting. This was amazingly similar to Dr. Jones' message, which emphasized the importance of using genomic approaches for finding genetic risk factors. Dr. Ludwig explained that as individual genetic architectures are different, variants can and do result in diverse and variable phenotypes. Technologies, such as SNP-arrays and Next-Generation Sequencing, are used to detect unique variants, but they must be used in concert with large cohorts to separate benign from causative variants.

Since 2009, there have been many GWAS studies identifying a number of CL/P loci, including one in a noncoding region at 8q24 by Dr. Ludwig's group, which another group later showed regulates *Myc* expression in the limb and face in mice. From these GWAS studies, currently 40 susceptibility regions have been identified, 39 of which are in noncoding regions, for CL/P. In contrast, for CPO, many fewer loci have been identified; one identified by Dr. Ludwig's group and others is *GRHL3*. To answer the question of whether genetic risk factors for CL/P and CPO overlap, studies from three different groups using different approaches (targeted replication assay, GWAS comparisons, and polygenic risk score analysis) concurred that variants of *FOXE1* are common to both CL/P and CPO. Currently, Dr. Ludwig is focused on using genetic variability to understand craniofacial biology by integrating GWAS data with functional datasets, using allelic series to identify candidate genes, performing functional assessments of noncoding variants, and using variants to understand environmental influences. Dr. Ludwig highlighted one recent study, performed in collaboration with Dr. Shelley Berger's group (University of Pennsylvania), in which they expressed *TP63* [involved in Ankyloblepharon–ectodermal defects-cleft lip/palate syndrome (AEC syndrome; OMIM 106260)] and *KLF4* in fibroblasts to identify epigenetic changes resulting from driving these cells down a keratinocyte pathway. When comparing RNA-seq data from the transfected cells to GWAS datasets, they found genes that are known to cause CL/P as well as others in CL/P loci not previously identified as causative. Analysis of enhancers in the *TP63/KLF4* dataset identified significant enrichment in CL/P risk SNPs, thus providing novel candidate SNPs for future study. Dr. Ludwig closed by encouraging a systems biology approach to identify and functionally characterizing genetic risk factors for craniofacial dysmorphologies as has been successful for orofacial clefting.

The SCGDB is greatly appreciative of the sponsorship of *Developmental Dynamics* to honor the exceptional contributions of our members.

4 | SCIENTIFIC SESSION I: MOLECULAR REGULATION OF CRANIOFACIAL DEVELOPMENT

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

In this session, new results on the molecules and pathways regulating craniofacial development were shared in the context of a variety of animal model systems.

Dr. Paul Kulesa (Stowers Institute for Medical Research) anchored the session by discussing his work in the chick embryo aimed at elucidating mechanisms controlling neural crest cell (NCC) invasion. This process of invasion allows NCCs at the front, or "leaders," to invade a target tissue, in part in response to the neural crest chemoattractant VEGF. Subsequently, NCCs at the back of the stream, or "trailers," gain directional input from the "leaders." Photoactivation experiments revealed that while NCCs move locally, there is no widespread rearrangement among them that occurs within the stream. Not surprisingly, "leaders" and "trailers" express a distinct repertoire of genes; however, "trailers" transplanted to the front of the invading NCC stream adopt the gene expression profile of "leaders" in response to the microenvironment and are able to migrate, revealing their plasticity. Moreover, NCCs migrate in the context of the mesoderm, which proliferates nonuniformly in space and time but maintains a constant cell density, indicative of tissue growth. NCCs, however, move faster and straighter than the mesoderm, with their active migration confined to specific zones by inhibitory molecules such as DAN. Intriguingly, the most invasive NCCs (or "trailblazers") possess a specific molecular signature, including the expression of *aquaporin1*, which encodes a water channel protein that localizes to the tips of NCC filopodia. Not surprisingly, knockdown of *aquaporin1* reduces NCC migration. Dr. Kulesa concluded his talk by presenting 10x single-cell genomics data that revealed conservation in gene expression in "trailblazers" from the various NCC streams entering the four branchial arches. These results highlight the importance of these "trailblazers" in pioneering invasion during migration into these different microenvironments and provide insight into mechanisms underscoring craniofacial patterning.

The second speaker of the session, Dr. M. Kathleen Pitirri (Pennsylvania State University), presented data uncovering new information about the role of Runt-related transcription factor 2 (RUNX2) and Osterix (Osx) in osteoblast cell development through the generation of two new mouse lines. The first line, *R2Tom*, contains an enhancer fragment from the human *RUNX2* gene and the *Hsp68* minimal promoter driving expression of the tdTomato reporter. This reporter labels early-stage osteoblast cells. This line was then crossed with *Osx-GFP*, generating a second new mouse line, *R2Tom;Osx-GFP*, that allows for detection of both *RUNX2* and *Osx* expression using different fluorescent reporters. Using the changing shape of the mouse limb bud, Dr. Pitirri established a novel staging method for mouse embryos, enabling staging within a window of +/− 2 h. This staging scheme was used to examine expression of *RUNX2* and *Osx* over developmental time within osteoblast cells forming the frontal and parietal bones and coronal suture. Using high-resolution fluorescence

microscopy in whole embryos, as well as examining sections of embryos, Dr. Pitirri demonstrated that *RUNX2* and *Osx* reporters are not coexpressed in osteoblast cells. These new mouse models provide powerful experimental tools for the continued study of dermal bone and cranial suture formation.

Next, Mr. David Tatarakis (University of California, Irvine) discussed his results regarding the developmental mechanisms that underlie the diversification of cranial NCC lineages using the zebrafish model system. To this end, NCCs were isolated by FACS using a photoconvertible *Sox10:EOS* transgene, and single-cell RNA sequencing was performed to identify the gene expression profiles of cells at distinct stages and locations in the embryo. By focusing on NCCs within the mandibular arch, Mr. Tatarakis demonstrated that the lineages present during the "middle" portion of NCC migration (around 18 hours postfertilization) first include progenitors that will form either skeletal or pigment cells, with other NCCs fated to become neurons or glia. These lineage decisions are mediated, in part, by Wnt signaling, as later shown through bulk RNA sequencing to identify gene expression profiles of cells having high or low levels of *Wnt* transcripts. By studying these novel Wnt targets in NCCs over developmental time, a more holistic view of Wnt signaling, and its effects on NCCs, has been acquired. These studies provide critical insight into developmental decisions that occur during craniofacial patterning and provide further evidence that Wnt signaling is an important regulator of these processes.

The role of alternative splicing during craniofacial development was addressed by Dr. Hong Li (University of Colorado Anschutz Medical Campus). Dr. Li and her colleagues used deep RNA sequencing to uncover transcripts in both ectodermal and mesenchymal tissues from the three facial prominences. These studies were carried out over three specific developmental stages to examine the dynamics of differential splicing. While Dr. Li's results revealed expected changes in gene expression over time, many genes were solely regulated by alternative splicing, highlighting the importance of splicing events to foster transcript diversity as the facial prominences form. Notably, exon skipping predominates with respect to methods of alternative splicing used in the embryo, with splicing changes between ectodermal and mesenchymal tissue layers, and over time, being more common than splicing changes noted among the facial prominences. In-depth analyses of the datasets were conducted to correlate expression of distinct RNA binding proteins with splicing events and to map the binding sites of molecules regulating splicing to intron–exon boundaries. Finally, gene expression studies through in situ hybridization and BaseScope were also presented to validate the datasets. Collectively, these findings further emphasize the importance of splicing during craniofacial development.

5 | SCIENTIFIC SESSION II: CRANIOFACIAL MORPHOGENESIS

5.1 | Chair: Dr. Samantha Brugmann (Cincinnati Children's Hospital Medical Center)

The second scientific session focused on discussing the forces that initiate and dictate the morphology of the craniofacial complex.

Dr. Ralph Marcucio (University of California, San Francisco), the immediate past president of SCGDB, led the session by discussing the extensive body of work his lab has generated over the last 20 years examining how Hedgehog (Hh) signaling drives facial morphogenesis. The Hh pathway has long been of interest to craniofacial biologists because of its association with human syndromes such as holoprosencephaly, but the mechanisms underlying the molecular control of midfacial development by Hh were unclear. Using various animal model systems and a series of intricate transplantation studies, Dr. Marcucio's laboratory identified a conserved Hh signaling center required for growth, patterning and morphology of the upper jaw, termed the frontonasal ectodermal zone (FEZ). Results from years of experimentation suggested a two-step model for *Shh* induction in the FEZ. First, Shh-dependent signals from the forebrain confer competence upon the facial ectoderm to express *Shh*. Second, NCCs, which migrate between the forebrain and facial ectoderm, induce *Shh* expression in the ectoderm. While initially identified as a domain of *Shh* expression in the frontonasal prominence initiated through transductive events between the forebrain, surface ectoderm and intervening NCCs, gain- and loss-of-function studies support a more complex scenario suggesting that the FEZ is not just a domain of *Shh* expression but a set of modules that have distinct regulation. For example, both BMP and FGF expression and activity in the NCC-derived mesenchyme are required for maintenance and expansion of *Shh* in the FEZ. Dr. Marcucio concluded his talk by presenting ongoing work examining cis-regulatory mechanisms involved in conferring *Shh* expression and activity in the FEZ and identifying additional transcription factors such as Pbx1 and Pbx3 that function to both activate and repress *Shh* expression in the FEZ. Together, these results represent a plethora of work at the molecular, cellular, and genomic levels geared toward understanding how Hh activity drives facial morphology.

Next, Dr. Kate Woronowicz (Boston Children's Hospital) presented her work geared toward unraveling genetic mechanisms that facilitate variability of craniofacial structures. To approach molecular mechanisms of variation, Dr. Woronowicz focused on a species of Dolly Varden char in Lake Kronotskoe that contains seven distinct "morphs" characterized by facial variation specific to the head, nose, and mouth. Each "morph" was analyzed via Phylo "mapping"—a technique that allows systematic comparison between evolutionary mutants and sister lineages. Fixation index analysis was subsequently used to quantify differences in allele frequency between morphs. By comparing a set of highly differentiated coding and noncoding variants between nosed-, head-, and mouthed-morphs, a number of interesting candidate genes were identified. *Anoctamin-1* (*ano1*), encoding a calcium-activated chloride channel that transports anions across epithelia, is differentially expressed in nosed-morphs and is implicated in mammalian nasal cancers. In zebrafish, *ano1* is expressed in nasal and oropharyngeal epithelia, supporting the hypothesis that candidates identified by these methodologies drive facial variation across species. In addition to *ano1*, variants in *setd5*, encoding a lysine methyltransferase for histones 3 and 4, were found to be exclusive to morphs with large mouths and long heads. Humans who are

haploinsufficient for *SETD5* present with micrognathia. Current studies are geared toward examining the functional role of genes like *ano1* and *setd5* in order to understand forces that drive naturally occurring variation that contribute to the etiology of human craniofacial malformations.

Ms. Aparna Baxi (George Washington University School of Medicine and Health Sciences) discussed her results surrounding various aspects of otic morphogenesis and forces underlying hearing loss through applying high resolution mass spectrometry. The rationale for such an approach centered on the observation that more common transcriptomic approaches do not always predict protein levels during development. To initiate the study, otic vesicles from a series of *Xenopus laevis* embryonic stages were collected, proteins were extracted and digested into peptides, and replicates were barcoded to allow for quantitation throughout the temporal course of development. Samples were fractionated and analyzed via liquid chromatography and mass spectrometry. Approximately 5000 proteins were identified, and six major patterns of protein dynamics were noted during otic morphogenesis. Interestingly, ECM and cytoskeletal proteins were identified as a major subset of proteins that increase in abundance as the inner ear develops, with the integrin pathway being overrepresented. From this dataset, the collagen-binding transforming growth factor beta-induced (Tgfbi) protein was identified as a candidate essential for otic development. Preliminary experiments knocking down Tgfbi in *Xenopus* embryos revealed a downregulation of several markers essential for otic development (*six1*, *dlx5*, *and pax2*), as well as an overall reduction in the size of the otic vesicle. Future studies will address the cellular mechanisms by which Tgfbi controls otic tissue differentiation.

Dr. Walid Fakhouri (University of Texas Health Science Center at Houston) presented the last talk of the session. Dr. Fakhouri's laboratory focuses on cell fate regulation of cranial NCC development and has long held an interest in the role of interferon regulatory factor 6 (IRF6) as it pertains to the onset of Van der Woude syndrome (VDWS). Previous studies from his laboratory had identified a rare human mutation in the enhancer element of *IRF6* that disrupts the cis-overlapping motifs that mediate binding of the transcription factors TP63 and TWIST1, and is etiologic for VWS. *Irf6*$^{+/-}$;*Twist1*$^{+/-}$ compound heterozygote mouse embryos presented with cleft palate, cranial skeletal defects, and holoprosencephaly, which suggested a genetic interaction between these two factors during craniofacial development. In a series of genetic, cellular, and biochemical experiments, Dr. Fakhouri's group uncovered a role for IRF6 and TWIST1 in neural tube and cranial NCC development and hypothesized a mechanism by which TWIST1 phosphorylation is critical for its ability to suppress E-cadherin and IRF6 in the epithelium, thus allowing for cranial NCC to transition to a migratory mesenchymal population. This epithelial-to-mesenchymal transition (EMT) occurs through the regulation of a number of cytoskeletal reorganization factors including SPWC1L and RHOA. Collectively, these findings establish a firm mechanism for VWS and elucidate possible areas of therapeutic intervention.

6 | SCIENTIFIC SESSION III: TRANSLATIONAL CRANIOFACIAL BIOLOGY

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

The third scientific session focused on studies examining the mechanistic basis behind craniofacial differences and the genes associated with these differences.

Dr. Juhee Jeong (New York University) began the session by discussing molecular mechanisms driving development of the calvaria. The skull is composed of bone plates (calvaria) and fibrous soft tissue (sutures), which allows the growth of the skull to accommodate the growing brain. It was believed that the frontal bones arise from the supraorbital mesenchyme (SOM) growing apically, rather than early migrating mesenchyme (EMM) moving basally from the apex. Previous studies illustrated that *Lmx1b* expression in EMM-derived cells prevents osteogenesis, as deletion of *Lmx1b* resulted in heterotopic osteogenesis in EMM-derived cells. To better assess cell fate of EMM-derived cells, RNA-seq of early EMM- and SOM-derived cells from E12.5 mouse embryos was used to identify the EMM-specific gene, *transgelin* (*Tagln*), which encodes a cytoskeletal protein in smooth muscle. Administering tamoxifen to *Tagln-CreER*;*R26R-YFP* mice at E10.5 and E11.5 revealed that by E16.5, a few labeled cells were observed in the meninges or dermis on the basal side. On the apical side, a few labeled cells were observed in the dermis and in the periosteal mesenchyme layer outside of the bone layer. By E18.5, Sp7 (bone) and YFP double-labeled cells were found close to the midsuture mesenchyme. At some places, YFP was present within the Sp7 cells (preosteoblasts); at other places, YFP-labeled cells were negative for Sp7 but were now positive for Osteopontin (mature osteoblasts). Thus, the working model is that EMM-derived cells at the dorsal midline undergo osteogenic differentiation to contribute to the apical growth of the frontal bone between E16.5 and E18.5. Further investigation of Lmx1B function has shown that it is downregulated in the apical mesenchyme from E16.5 onward, suggesting that the antiosteogenic effect of Lmx1B is transient. Thus, while early *Lmx1b* expression blocks ossification of EMM-derived cells, its downregulation allows EMM-derived cells to contribute to the late growth of the apical end of the frontal bone. These studies illustrate that Lmx1B is crucial for ensuring that premature fusion of skull bones (craniosynostosis) does not occur.

The second speaker of the session was Dr. Christian Bonatto (Cincinnati Children's Hospital Medical Center), who presented his work detailing the mechanism behind one form of ciliary micrognathia. Cilia are microtubule-based organelles that are crucial for many developmental processes. Any disruption to ciliary extension or activity results in a disease called a ciliopathy. Of ciliopathies, 30% can be primarily classified by their craniofacial phenotype. One such ciliopathy is Oral-Facial-Digital syndrome 14 (OFD14), which is caused by mutations in a gene encoding the basal body protein C2CD3. To study the etiology of micrognathia in OFD14, the avian *talpid*^{2} mutant was used,

as *talpid*^{2} mutants contain a deletion in *C2CD3* and phenocopy OFD14 individuals. By HH36, *talpid*^{2} embryos had decreased mandibular mineralization and volume. Bulk RNA-seq identified genes involved in cell cycle progression, osteoblast differentiation, and ossification as those differentially expressed in *talpid*^{2} embryos. Further analyses revealed that unchecked cell cycle progression of NCCs in the mandibular prominence resulted in premature mesenchymal condensation and skeletal differentiation. These results were validated via the observed downregulation of *Hand2* and subsequent upregulation of *Runx2* (whose function is negatively regulated by Hand2 during mandible ossification). Interestingly, the increase in skeletal progenitors did not correlate with increased mandible ossification, as osteoblast maturation was arrested prior to completion. Furthermore, increased tartrate-resistant acid phosphatase (TRAP) staining, which is a marker of osteoclasts, suggested that excessive bone resorption also contributed to micrognathia. These results illustrate that ciliopathic micrognathia likely occurs due to insults at multiple stages of skeletal development.

Next, **Mr. Waheed Awotoye** (**University of Iowa**) presented his studies identifying a new risk locus associated with nonsyndromic orofacial clefts (NSOFCs) in Africans through the analysis of genome-wide gene by sex (GxSex) interactions. NSOFCs account for 70% of all OFCs, but with a number of genetic and environmental interactions involved, GWAS studies have only explained ~25% of the heritability of NSOFCs. Furthermore, NSOFCs are more common in females, though the basis for this is not known. To address the question of gene–sex interactions, 1019 NSOFC cases and 2159 controls were recruited from Ethiopia, Ghana, and Nigeria. Common SNPs and rare variants were genotyped, and an additive logistic model was used to examine the joint effects of genotype by GxSex interaction effects compared to GxSex interactions only. One SNP on chromosome 8 was identified; using the SysFace bioinformatics program, the *zinc finger-DHHC-palmitoyltransferase type 2* (*ZDHHC2*) gene was identified as being enriched in the frontonasal and maxilla regions of developing mouse embryos. ZDHHC2 catalyzes palmitate addition to proteins and influences aspects that include cell motility and morphology. Web-based interaction analysis suggests that ZDHHC2 interacts with MAFB, itself associated with NSOFCs. These studies illustrate that GxSex studies can be used to identify candidate NSOFC genes that can impact future translational research approaches to treating NSOFCs.

In the last talk of the session, Dr. Irfan Saadi (University of Kansas Medical Center) presented his work on the role of SPECC1L, a cytoskeletal scaffolding protein, in palatal shelf fusion and neural tube closure. Both of these developmental events require movement and fusion of embryonic tissues. SPECC1L is a large protein with a C-terminal calponin homology binding domain and eight coiled-coil domains, with human *SPECC1L* variants associated with several craniofacial dysmorphology syndromes. Most variants cluster in the second coiled-coil domain (CCD2), which drives association with acetylated microtubules. As multiple truncation mouse mutants of *Specc1L* do not result in cleft palate or exencephaly, an in-frame deletion of CCD2 (*Specc1L-ΔCCD2*) was created, with these mutants showing both cleft palate and exencephaly. All embryos had a delay in palatal shelf elevation, although this delay was almost completely attributed to female *Specc1L-ΔCCD2* embryos due to the fact that the normally slower shelf elevation in control female versus male embryos was more sensitive to the *ΔCCD2* mutation. Mouse embryonic palatal mesenchyme cells were then used to show that SPECC1L colocalization with F-actin was disrupted in *Specc1L-ΔCCD2* mutants, while nonmuscle myosin II localization was aberrantly confined to the cell periphery. These results contributed to formulating a model in which SPECC1L modulates actomyosin forces during embryonic tissue movement and fusion. Further analysis of *Specc1L-ΔCCD2* mutants may help elucidate the basis of other developmental defects resulting from tissue fusion failure.

7 | SCIENTIFIC SESSION IV: SIGNALING DURING CRANIOFACIAL DEVELOPMENT

7.1 | Chair: Dr. Jean-Pierre Saint-Jeannet (New York University)

The last scientific session of the meeting focused on the signaling pathways regulating craniofacial development.

The plenary presentation by Dr. Joy Richman (University of British Columbia) discussed the role of the noncanonical Wnt/planar cell polarity (PCP) pathway in the context of autosomal dominant Robinow Syndrome (RS), a human condition characterized by craniofacial and limb abnormalities. The major genes linked to this disease impact the noncanonical Wnt pathway, including genes encoding the adaptor proteins DVL1 and DVL3, the Wnt receptor FZD2 and the WNT5A ligand. To understand this syndrome, the Richman lab uses local transgenesis, expressing the human mutant genes in chicken and *Drosophila* embryos and analyzing the morphological, cellular, and signaling consequences. Misexpression of WNT5A variants in the face of chicken embryos led to a shortening of the beak and randomization of chondrocyte polarity within Meckel's cartilage. In the limbs, misexpression of WNT5A variants resulted in shorter limb cartilage presumably due to aberrant stacking of chondrocytes. These defects were associated with enhanced noncanonical Wnt signaling. Similarly, expression of DVL1 variants in the limbs resulted in shorter limbs, thicker cartilages, and loss of chondrocyte polarity. To understand the mechanism underlying this phenotype, Dr. Richman and colleagues turned to the *Drosophila* wing bristles, a bona fide system to analyze the PCP pathway. Expression of human DVL1 variants caused distinct polarity defects of the wing bristles, including misoriented bristles and ectopic edge bristles. Targeting human transgenes to specific segments of the wing using Gal4 drivers suggested that the mutant proteins interfere with BMP and Hedgehog signaling pathways, pointing to a possible cause for loss of cell polarity. This work highlights the importance of animal models to further our understanding of rare genetic disorders such as autosomal dominant RS.

The next speaker, Dr. Michael Piacentino (California Institute of Technology), discussed his work investigating the impact of changes

in plasma membrane dynamics and lipid composition on cranial neural crest EMT in chicken embryos. Experiments using live imaging to measure plasma membrane dynamics in chick neural crest explants revealed that following EMT, NCCs have increased membrane fluidity compared to their premigratory precursors. Interestingly, neutral Sphingomyelinase 2 (nSMase2), the enzyme that converts sphingomyelin into ceramide, is strongly enriched in both premigratory and migratory neural crest. Upon nSMase2 knockdown, migratory NCCs have reduced membrane fluidity and decreased directional cell migration. In nSMase2 morphant embryos, neural crest EMT was impaired, and these cells had reduced *Snai2* and *Sox9* expression and increased *Cad6B* expression, a gene expression profile characteristic of a premigratory neural crest state. Using reporter constructs, Dr. Piacentino provided evidence that nSMase2 is also required to activate Wnt and BMP signaling through the formation of endosome-specific signaling complexes. Ceramide is known to induce endocytosis, and nSMase2 overexpression in NCCs resulted in increased endocytosis presumably through ceramide production. Conversely, the expression of a dominant-negative Dynamin1, which interferes with the endocytic pathway, phenocopied the nSMase2 knockdown EMT neural crest phenotype and led to reduced Wnt signaling activity. Altogether these results indicate that subtle changes in lipid composition can alter the biophysical properties of the plasma membrane and initiate a complex cascade of cellular events that regulate cranial neural crest EMT and directional cell migration.

Next, Ms. Zuzana Vavrusova (University of California San Francisco/ Universite Paris-Saclay) presented her studies investigating the role of Shh signaling in the control of jaw size across avian species. Shh is expressed in the pharyngeal endoderm, and its deregulation results in atypical mandible size. Ms. Vavrusova hypothesized that Shh activity within the mandibular NCC-derived mesenchyme (NCM) is species specific and regulates cell proliferation and jaw size. Comparative analysis of duck and quail embryos previously established that duck embryos start with more NCC-derived mesenchyme in their mandibular arch and have distinct proliferation dynamics when compared to quail embryos. To test whether the NCC-derived mesenchyme response to Shh signaling is species specific, Ms. Vavrusova used an in vitro mandible culture assay and reverse transcriptase-polymerase chain reaction to measure *Ptch1* and *Gli* activation, two Shh targets. Treatments of dissected mandibular primordia with increasing doses of recombinant Shh indicated that duck and chick explants had a greater response to Shh than quail explants. Another Shh target, *Gas1*, a negative regulator of cell cycle, is expressed at 25–75 times higher levels in duck mandible primordia than in chick and quail mandibles. *Gas1* is also more sensitive to Shh manipulation in the quail mandible compared to duck and chick. Interestingly, in duck, Gas1 overexpression in vivo is sufficient to significantly reduce the size of the mandibular NCC-derived mesenchyme population. These results suggest that differences in the mandibular NCC-derived mesenchyme response to Shh signaling may regulate jaw size during development and may underlie species-specific variations in jaw size.

For the last talk of the session, Dr. Radhika Atit (Case Western Reserve University) discussed experiments aimed at dissecting the mechanisms by which calvarial osteoblasts expand apically from the supraorbital mesenchyme (SOM) during mouse skull development. Fibronectin protein is expressed in a gradient toward the apex of the skull as SOM cells initiate their expansion and is therefore a good candidate to regulate this process. Consistent with this possibility, conditional deletion of Fibronectin disrupted the apical expansion of calvarial bones, without altering osteoblast differentiation and proliferation. Dr. Atit and colleagues hypothesized that Wnt signaling may regulate Fibronectin expression in this region, as SOM cells express several components of the noncanonical Wnt/PCP pathway and are polarized. Mouse embryos with SOM inactivation of *Wntless*, which is required for secretion of all Wnt ligands, have reduced Fibronectin protein expression and show impaired bone apical expansion. These results suggest that noncanonical Wnt ligands in the SOM regulate the expression of Fibronectin, which provides long-range cues for directional collective migration of the cranial mesenchyme toward the apex of the skull. Future studies will map basoapical differences in SOM cell movement that may participate in the initial migration and apical expansion of the calvarial bone primordia.

8 | POSTER SESSIONS

8.1 | Organizer: Dr. David Clouthier (University of Colorado Anschutz Medical Campus)

Each day ended with a 4-hour "poster" session, in which faculty, postdocs, and students presented their poster abstracts in the form of an 8-minute Zoom presentation. Winners of the poster presentation awards, sponsored by the American Association for Anatomy, were as follows: Drs. Carrie Leonard (1st), Michi Kanai (2nd) and Soma Dash (3rd) in the postdoctoral fellow category; and Lauren Bobzin (1st), Kimberley Diaz-Perez (2nd), and Beatriz Ibarra (3rd) in the graduate student trainee category.

9 | SUMMARY

In summary, the 43rd annual meeting of the Society for Craniofacial Genetics and Developmental Biology brought together a diverse group of scientists to present and discuss cutting edge approaches to better our understanding of craniofacial development and alterations that result in pathogenesis. The meeting continues to be an outstanding forum for the craniofacial sciences and a welcoming environment for all researchers regardless of career stage, gender, or background. We look forward to convening for the 44th annual meeting of the SCGDB. Due to the ongoing COVID-19 crisis and its associated uncertainties, this meeting will be held virtually on October 18–19th, 2021.

ACKNOWLEDGMENTS

We thank Dr. Amy Merrill-Brugger for critically reading the draft manuscript. None of the authors have a conflict of interest to declare.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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How to cite this article: Brugmann S, Clouthier DE, Saint-Jeannet J-P, Taneyhill LA, Moody SA. The society for craniofacial genetics and developmental biology 43rd annual meeting. *Am J Med Genet Part A*. 2021;1–8. https://doi.org/10.1002/ajmg.a.62150