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CONFERENCE REPORT

The Society for Craniofacial Genetics and Developmental Biology 41st Annual Meeting

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

KEYWORDS

craniofacial, developmental biology, genetics

1 | OVERVIEW

Blue skies and scenic views welcomed participants to the 41st Annual Meeting of the Society of Craniofacial Genetics and Developmental Biology (SCGDB) held in La Jolla, California, at the Sanford Consortium for Regeneration at the University of California, San Diego, on October 16, 2018. Once again, the SCGDB meeting preceded the annual meeting of the American Society for Human Genetics and allowed for interactions among multiple investigators and trainees from around the world, all sharing a common interest in craniofacial biology. The meeting consisted of a dedicated clinical session, which, together with basic research sessions, provided insight into craniofacial genetics, development, disease, and repair. Invited speakers included Drs. Timothy Cox, Martine Dunnwald, Marilyn Jones, Ophir Klein, Alysson Muotri, Drew Noden, Paul Trainor, and Stephen Twigg. These speakers, along with oral presentations chosen from submitted abstracts, resulted in a day of outstanding science. In addition, a large poster session was held to further showcase the work being done in the craniofacial development and disease fields. Importantly, speakers included graduate students and postdoctoral fellows, highlighting a key objective of the Society—to promote its junior members and create an inclusive atmosphere that facilitates stimulating scientific discussions. In accordance with this, travel awards in the form of poster prizes were made available to both postdoctoral scientists and graduate students. Postdoctoral scientist travel awards were made through the support of the American Association of Anatomists, which also sponsored the evening poster session, and *genesis*, *The Journal of Genetics and Development*. Student travel awards were provided by SCGDB and *genesis*. In the postdoctoral scientist category, D'Juan Farmer (University of Southern California) received the first-place award, with second and third place going

to Erica Hutchins (Caltech) and Andrew Cornith (University of Massachusetts, Amherst), respectively. Kristin Watt (Stowers Institute for Medical Research) received the fourth-place award (sponsored by *genesis*). In the student category, first place went to Olivia Hung-Jhen Chen (University of Southern California), second to Karla Terrazas-Falcon (Stowers Institute for Medical Research), and third to Dion Giovannone (University of Southern California), with the *genesis* award for fourth place going to J. David Aponte (University of Calgary). Congratulations to all for outstanding presentations. The meeting also received generous support from Illumina.

Here, we present a brief overview of the 2018 meeting. The 42nd annual meeting of the SCGDB will be held October 14–15, 2019, in Houston, Texas, immediately preceding the American Society for Human Genetics annual meeting.

2 | PLENARY SESSION I: GENETICS OF CRANIOFACIAL MORPHOLOGY

This session focused on the power of genomics, biochemistry, cell biology, and other experimental tools to reveal genes associated with craniofacial anomalies. The session began with a presentation by one of the plenary speakers, **Stephen Twigg** (Weatherall Institute of Molecular Medicine), who reviewed the various genes associated with craniosynostosis and then described the work of his group in characterizing two new genes, *DHRS3* and *BCL11B*, that play roles in this disease. *DHRS3* was discovered through dense SNP-chip analyses of affected individuals, which revealed a deletion in exon 1 that still left the coding region intact. DHRS3 converts all-trans retinaldehyde to retinol (vitamin A), and, interestingly, other mutations in the retinoic acid pathway lead to craniofacial anomalies, as noted in knockout mouse models. Individuals homozygous for this deletion possess little *DHSR3* mRNA, and in the absence of DHRS3, retinol is decreased. A de novo missense mutation in a second gene, *BCL11B*, was identified to be a dominant mutation causing craniosynostosis. *BCL11B* encodes a transcription factor that functions in diverse contexts, including the skin, neural development, immune system, and the craniofacial skeleton. Interactome analysis revealed a role for BCL11B in the NuRD and Polycomb (PRC2) repressor complex, with these interactions lost upon mutation. Knockout of *BCL11B* in mice gave a craniosynostosis phenotype, and knock-in of this mutation via CRISPR resulted in homozygous lethality, with both heterozygous and homozygous mice possessing coronal suture defects. Interestingly, Dr. Twigg showed that loss of *BCL11B* led to an increase in RUNX2, which is responsible for maintaining cells in a pre-osteogenic state.

The next speaker, **Diana Rigueur** (University of Southern California), outlined her findings on characterizing the etiology of Bent Bone Dysplasia Syndrome (BBDS) using a mouse conditional knock-in allele of a mutation in *FGFR2* (M391R) that recapitulates the human disease. BBDS-afflicted individuals possess nuclear-localized FGFR2 due to the above mutation within the transmembrane domain of FGFR2, and have several skeletal defects, including loss of mineralization of the calvarium and increased proliferation in the long bones. Once localized to the nucleus, FGFR2 activates p53 and precludes appropriate osteoblast differentiation, thereby biasing cells towards self-renewal. To model this disease, a *Wnt1-Cre; FGFR2*^{M391R}/+ mouse was generated, resulting in FGFR2 localization to the nucleus as noted in humans with BBDS. Moreover, microCT analyses revealed that mice develop craniosynostosis and possess defects in joint establishment. This now provides a powerful model to elucidate the function of nuclear FGFR2 in skeletogenesis.

James Cheverud (Loyola University, Chicago) then followed with a talk describing the use of mathematical modeling to further refine the relationship between developmental and phenotypic variance. Using the phenotypic changes in midfacial morphology and variations in *FGF8* and *SHH* as an example, Dr. Cheverud showed how a readout of "wildtype" is associated with relatively flat regions along a von Bertalanffy curve. Interestingly, changes in *FGF8* expression here have minimal effect on outward phenotype and, as such, do not generate variation. Conversely, when FGF8 and SHH levels drop to 40–50% of wildtype, the readout falls along a very steep portion of the curve such that small changes in gene expression yield more dramatic phenotypic variation in midfacial morphology. These results highlight the dynamic interplay between gene expression levels and phenotypic output.

Stanley Kanai (University of Colorado, Anschutz Medical Campus) presented data revealing a new mutation in *endothelin receptor type A* (*EDNRA*), p.Glu381Pro, that is associated with various craniofacial and heart defects in which the affected individual died 10 hr after birth. This mutation occurs in the cytoplasmic domain of EDNRA, which normally functions in the recruitment of G proteins. In order to investigate the intracellular signaling events downstream of EDNRA, particularly in the context of the cytoplasmic tail and activation of G proteins, a cell-based bioluminescence resonance energy transfer (BRET) assay was developed. Using this approach, the p.Glu318Pro mutation in EDNRA was demonstrated to be a loss of function due to the inability of the mutant receptor to recruit and activate G proteins. This technique now provides a powerful means by which to rapidly screen mutant receptors and assess function, lending insight into disease.

The last presentation of this session, given by **J. Robert Manak** (University of Iowa), highlighted results from a collaborative effort aimed at identifying novel craniofacial patterning genes through analysis of copy number variation in cleft lip and palate cases. Through array-based Comparative Genomic Hybridization of a population of 800 Filipinos, eight rare disease-causing alleles were identified. These genes were then validated in fish and frog, identifying normal expression patterns and then performing CRISPR-mediated gene editing to look at phenotypes. Six of the candidate genes possess craniofacial dysmorphologies in fish as well as frog upon their mutation, including aberrant mouths, underdeveloped eyes, and clefting. Intriguingly, three of these genes, *Radil*, *Ric1*, and *Cobll1*, are involved in Rho GTPase signaling and are expressed within cranial neural crest cells and/or the regions through which they migrate, lending further credence to their function in controlling craniofacial development.

3 | WORKSHOP: CLINICAL GENETICS AND PATIENT CARE

The Clinical Genetics and Patient Care workshop was co-chaired by **Marilyn Jones** (University of California, San Diego) and **Ophir Klein**

(University of California, San Francisco). Dr. Klein began with an introduction to the evaluation and testing process in clinical craniofacial genetics. He reviewed the composition of a multispecialty craniofacial team and the importance of team care, as well as potential medical treatments for Mendelian birth defect syndromes. He then discussed ongoing research that combines basic science with a human clinical problem: ectodermal dysplasia. Mutations in Ectodysplasin1 (EDA1) lead to defects in ectodermal appendages such as sweat glands, hair, and teeth. Prenatal EDA1 replacement in a mouse model abrogates phenotypes, and ongoing clinical trials have provided encouraging results. Dr. Klein closed by presenting preliminary data implicating EDA1 in the evolution of tooth morphology. Dr. Jones discussed in more depth how clinicians apply genetics to advance patient care. She examined the main classes of conditions seen by craniofacial geneticists, including orofacial clefting, craniosynostosis, and ocular hypertelorism. She discussed some of the testing modalities employed clinically and current clinical interventions. Finally, **Amanda Gosman** (University of California, San Diego) covered the approaches that plastic surgeons utilize to treat patients with craniofacial defects. Her talk focused on the use of distraction osteogenesis in craniofacial surgery, a procedure that was initially developed for use in long bones but has since been co-opted for use in the craniofacial complex. Recent advances in adapting this approach to lengthening the mandible or the maxilla, and for correcting different types of cranial vault and midface abnormalities, were presented.

4 | PERSPECTIVE: EVOLVING PERSPECTIVES ON CRANIOFACIAL NEURAL CREST

Drew Noden, Professor Emeritus of Anatomy and Embryology at Cornell University, gave a captivating lecture entitled, "*Evolving Perspectives on Craniofacial Development*." Drew's primary aim was to cover the life and contributions of Julia B. Platt to the field of developmental biology and address the impact Julia has had on our current manner of thinking about the craniofacial complex. In a way, Drew told two stories simultaneously. One was about Julia as a groundbreaking and remarkably skilled scientist who was ahead of her time in terms of her ideas and the embryological discoveries that she made in the late 1800s. The other parallel story concerned an unconventional, unintimidated, and unabashedly strong woman working in an age when male chauvinism and the old-boys network was rigidly intact. Drew emphasized how Julia trained at some of the best institutions and interacted with many of the greatest minds in embryology and anatomy such as Howard Ayers in the "Annex" at Harvard (where women were allowed). This impressive list also included E. B. Wilson at Bryn Mawr College, Charles Whitman at the Marine Biological Laboratory in Woods Hole (where apparently she was the subject of unwelcomed practical jokes), Anton Dohrn and Hans Driesch at the Zoological Station in Naples, Karl Wilhelm von Kupffer and Richard Hertwig at the University of Munich, Charles Davenport and H.V. Neal at Radcliffe College, and Robert Wiedersheim and August Weismann at the University of Freiberg, which is from where Julia received her PhD.

In characteristic style, Drew mixed humor (or noble attempts thereof), fascinating history, engaging storytelling, and beautiful slide illustrations, with serious science and rigorous data. He called "Miss Platt" an unintended provocateur whose pioneering studies were heretical to the dogma of the day. In particular, Julia's 1893 publication on the "ectodermic" contributions (i.e., the cranial neural crest) to the head cartilages of amphibians was fiercely rejected by proponents of the germ layer theory (for which there were many in that era). Julia also confidently interjected herself directly into the highly contentious debates on head segmentation with her 1890 work on the anterior head cavities in sharks. Among other things, Julia argued for a unifying and conserved plan for head segmentation, the conservation of muscle origins among vertebrates, the ability of cells to migrate long distances, and the shared fates of different germ layers. So beyond simply being an outspoken woman, Julia was also speaking about subjects that many of her male colleagues found objectionable, and thus according to Drew, Julia developed a reputation and became ostracized. In the end (and only 1 year after completing her PhD in 1898), Julia rejected this harsh and narrow-minded culture, left science, and moved to Pacific Grove in California. Here, Julia continued making valuable contributions such as being elected mayor in 1931 at age 74. Julia died in 1935, but her legacy lives on and in many ways has clearly had a big impact on the intellectual journey of Drew Noden himself. As evidenced by the arc of Drew's career and the content of his lecture, Drew's own seminal experimental work on the lineages and fates of the craniofacial mesenchyme in birds and his oftencited schematics on the general organization of the vertebrate head owe much to the efforts of Julia Platt (and Drew seemed very grateful).

5 | PLENARY SESSION 2: CELL BIOLOGY AND CRANIOFACIAL DEVELOPMENT

This plenary session illustrated the power of different model systems in elucidating molecules and pathways critical for craniofacial development. The session commenced with a plenary talk by **Timothy Cox** (University of Missouri-Kansas City), who discussed studies characterizing the intersection between maternal diet and genes and its role in the presentation of nonsyndromic cleft lip/palate (NS-CL/P). The inherent clinical variability observed with NS-CL/P suggests that other factors, such as nutrition, could be at play. To investigate this variability further, Dr. Cox's group used the mouse model to determine whether embryos possessing specific genetic risk factors for NS-CL/P were hypersensitive to changes in the bioavailability of maternal vitamin A, which on its own is a risk factor for orofacial clefts. Using the *Rbp4* knockout mouse that is normally healthy in the presence of vitamin A supplementation, Dr. Cox showed that embryos carrying a NS-CL/P susceptibility gene such as *Wnt9b*, together with a vitamin A-insufficient diet, resulted in an increased incidence of clefts, primarily of the lip and alveolus, when compared to those on a vitamin A-sufficient diet, pointing to a role for vitamin A in modulating the sensitivity of certain genotypes to the development of clefts. These results suggest that maternal dietary supplementation with vitamin A during pregnancy may be useful to reduce the incidence and/or severity of clefts, in an analogous fashion to the use of folic acid to prevent neural tube defects.

In the second plenary talk of this session, **Martine Dunnwald** (University of Iowa) described her work on Interferon Regulatory Factor 6 (IRF6), a transcription factor that functions during wound healing and palatogenesis. Interestingly, individuals with van der Woude syndrome, which is characterized by orofacial clefting, possess mutations in *IRF6* and have increased wound healing complications after surgical cleft repair. To gain more insight into IRF6 function, Dr. Dunnwald used *Irf6*-deficient mouse keratinocytes and embryos and found that *Irf6*-deficient cells migrate more slowly, have a decreased path length, and lack directionality, all of which contribute to their inability to close a wound. The molecular bases of this phenotype can be attributed, in part, to the presence of increased filamentous actin and an increase in RhoA GTPase activity. Moreover, the skin of *Irf6*-deficient mice ectopically expresses E-cadherin in the uppermost layer, where it is normally absent. *Irf6*-deficient keratinocytes also possess alterations in E-cadherin distribution and smaller lamellipodia, and are not polarized, further underscoring how two seemingly different biological phenomena, wound closure and palatogenesis, can be controlled by the activity of the same protein.

Continuing with the mouse model, **Brenna Dennison** (University of Colorado, Anschutz Medical Campus) presented her findings on the role of RNA splicing downstream of Pdgfra signaling during mouse development. Mutations in *PDGFRA* are associated with cleft lip/palate in humans, and the mouse knockout recapitulates these phenotypes, as do disruptions to PI3K and phospho-Akt signaling. A mass spectrometry-based phosphoproteomics screen was carried out using primary mouse embryonic palatal mesenchyme cells to identify protein binding partners of phospho-Akt, many of which included proteins involved in RNA splicing. Three of these RNA splicing proteins, Srsf3, Rbmx, and Roa1, were confirmed to be bona fide interactors with phospho-Akt and, interestingly, are expressed in the maxillary processes and palatal shelves of mid-gestation mouse embryos. To provide insight into transcripts alternatively spliced upon Pdgfra signaling, RNA-sequencing analysis was performed on wildtype and *Pdgfra* mutant embryos. This method identified transcripts differentially spliced in wildtype versus mutants, including those involved in controlling proliferation and/or craniofacial development, such as *Smad7*.

Marshall Lukacs (Cincinnati Children's Hospital Medical Center) then spoke about the requirement for glycophosphatidylinositol (GPI) biosynthesis in craniofacial development. A forward genetic screen identified a hypomorphic allele of a gene called *post-GPI attachment to proteins-2* (*Pgap2*), with mice possessing a bilateral cleft lip due to apoptosis of neural crest cells in the first branchial arch, along with neural tube defects. Pgap2 plays a required role in GPI anchor lipid maturation and is expressed broadly in early mouse embryogenesis but is enriched in the first branchial arch, as demonstrated through a *Pgap2-lacZ* gene trap. Intriguingly, other GPI biosynthesis enzymes are expressed in this region as well. One crucial GPI-anchored protein, the folate receptor, is required for neural tube closure and cranial neural crest survival, with some mouse mutants in this gene exhibiting cleft lip. Notably, supplementation of *Pgap2* mutants with folinic acid rescued the cleft lip phenotype by increasing cell survival. To investigate the cell autonomous requirement for GPI biosynthesis in cranial neural crest cells, a mouse model was generated in which neural crest cells are devoid of all GPI-anchored proteins due to deletion of the *phosphatidylinositol glycan anchor biosynthesis, class A* (*Piga*) gene, which encodes the first enzyme in GPI biosynthesis. These mice exhibit midline cleft lip and palate, likely due to death of neural crest cells, further underscoring the importance of GPI biosynthesis during development.

Karyn Jourdeuil (University of Maryland, College Park) discussed her results addressing a role for gap junctions during the formation of cranial neural crest cells. Gap junctions are comprised of proteins called Connexins, the most predominant being Connexin 43 (Cx43). Interestingly, mutations in Cx43 lead to oculodentodigital dysplasia, a disease characterized, in part, by craniofacial defects. Analysis of Cx43 in cranial neural crest cells revealed robust expression in premigratory neural crest cells, both prior to and during their epithelial-to-mesenchymal transition (EMT), along with expression in migratory neural crest cells. By injecting Calcein-AM, a dye that only passes through cells via gap junctions, into premigratory cranial neural crest cells, followed by live imaging, Dr. Jourdeuil showed that this dye could be transferred across the premigratory neural crest cell population. Moreover, Cx43 depletion experiments indicated a dependence on Cx43-containing gap junctions to transfer Calcein-AM between migratory neural crest cells cultured ex vivo. In keeping with this finding, chemical inhibition of general gap junction function, but not formation, abrogated neural crest cell EMT and/or migration in a reversible manner. Furthermore, depletion of Cx43 from premigratory neural crest cells in vivo altered the expression of some neural crest cell markers but, interestingly, did not overtly affect EMT. Altogether, these results implicate gap junctions in mediating communication between cranial neural crest cells.

The final speaker in the session, **Rulang Jiang** (Cincinnati Children's Hospital Medical Center), described the results of a single cell RNA-sequencing screen of the mandibular component of the first pharyngeal arch in E10.5 mouse embryos. Surprisingly, a high number of imprinted genes were shown to be differentially expressed throughout the mandibular arch. Many genes are expressed only from the paternal or maternal allele in mammals, due to the epigenetic regulatory mechanism of genomic imprinting, and dysregulation of imprinted genes has been associated with multiple human disorders such as Beckwith-Wiedemann, Russell-Silver, Kagami-Ogata and Temple syndromes, several of which exhibit craniofacial malformations such as micrognathia. However, little is known about the specific roles of imprinted genes in craniofacial development. Dr. Jiang showed that many of the imprinted genes are clustered in specific chromosome locations, and verified by in situ hybridization that over 20 imprinted genes are differentially expressed in the distal, proximal, rostral, and/or caudal domains of the facial prominences and branchial arches during early craniofacial development. Further investigation of the roles of these imprinted genes and the imprinted gene network during mandibular development may heighten our understanding of epigenetic regulation of craniofacial development and the pathogenesis of congenital disorders.

6 | PLENARY SESSION 3: MODELS OF DISEASE AND DEVELOPMENT

This plenary session provided several examples of model systems that are being used to decipher the underlying cellular, molecular and genetic causes of human syndrome craniofacial phenotypes. The

session began with a plenary talk by **Paul Trainor** (Stowers Institute for Medical Research), who discussed craniofacial syndromes that involve disruption of neural crest development—the so-called neurocristopathies. Because cranial neural crest cells are the progenitors of cranial bones, they frequently are involved in more than 700 distinct craniofacial syndromes. Treacher Collins syndrome, which is characterized by hypoplasia of the zygomatic complex and cleft palate, results primarily from mutations in *TCOF1*, which codes for a nuclear phosphoprotein called Treacle. In collaboration with the Michael and Jill Dixon (University of Manchester), a mouse model was developed that shows the same phenotypic variability as observed in human patients. Nulls are lethal at implantation whereas heterozygotes show cranial hypoplasia by E17.5. Various labeling studies showed that the neural plate suffers from a wave of apoptosis that leads to about a 30% reduction in neural crest cells that manage to migrate appropriately. Mass spectrometry analysis identified several proteins associated with ribosome biogenesis that bind to Treacle. In *Tcof1* mutants, there is a deficit in the number of ribosomes in all tissues and an upregulation of p53 targets and cell cycle genes. By reducing the levels of p53 in *Tcof1* heterozygotes, apoptosis levels decrease, neural crest numbers increase and the animals survive to adulthood and are fertile. The mass spectrometry analyses also revealed two other proteins (POLR1C and POLR1D) that are mutated in about 10% of Treacher Collins patients. Knocking out the genes that encode these proteins, which are subunits of RNA polymerase complexes, also leads to craniofacial dysmorphologies in zebrafish that can be rescued by p53 reduction. An associated protein, POLR1A, is mutated in acrofacial dysostosis patients, who have phenotypes that overlap with Treacher Collins. *Polr1a* mutant fish also have craniofacial hypoplasia that can be partially rescued by reducing p53 levels. Because all of these proteins are ubiquitously expressed, why are cranial neural crest specifically affected? Experiments suggest that neural crest cells are translating proteins at a higher rate than other cells and therefore may be particularly susceptible to reductions in the RNA biosynthetic machinery.

M. Chaise Gilbert (University of Massachusetts, Amherst) then spoke about the role of Crocc2, a key component of the rootlet complex of primary cilia in craniofacial defects. Primary cilia are well known to play important roles in craniofacial development, and mutations in a large number of the proteins that contribute to this signaling complex result in craniofacial dysmorphologies. To understand the function of Crocc2, Mr. Gilbert mutated its gene in zebrafish and performed detailed morphometric analyses of the cranial skeletal elements. A ventral view of the cranial skeleton revealed that the cartilages in nulls and heterozygotes have very different developmental trajectories compared to wildtype. The mutants are particularly dystrophic at 7 days postfertilization, the time at which they begin to feed. Analyses of elements from a lateral view revealed no anatomical differences in shape trajectories except at Day 12 for the nulls; this resolves by Day 18. From these measurements, the working hypothesis is that the rootlets of primary cilia regulate early developmental processes in a function unrelated to cilia signaling, but may involve interactions with the cytoskeleton.

Continuing with the zebrafish model, **Joanna Smeeton** (University of Southern California) discussed her work to model osteoarthritis of the jaw joint. This is a degenerative joint disease, which often follows joint trauma, for which there is no current treatment in humans. In fish, transection of the interopercular (IOP) ligament, which is required for generating bite force, leads to jaw joint degeneration. But, unlike in humans, in fish the damaged joint can regenerate in about 2 weeks. Dr. Smeeton is using this model to discover genes that are responsible for regeneration in the fish in hopes that this information will be useful in studies of the human condition. Using different mutant and reporter fish lines, Dr. Smeeton found that the expression of Sox10, a neural crest cartilage progenitor marker, and Scleraxis, a transcription factor involved in tendon development, are required for regeneration. Current research is focused on analyzing the roles of a number of potential genes identified by RNA-sequencing of samples dissected from the Day 3 regenerating jaw joint and single cell RNA-sequencing of Sox10-positive cells.

Everett G. Hall (University of Kansas Medical Center) next discussed his group's work on understanding the role of SPECC1L (Sperm antigen with calponin homology and coil-coiled domains 1-like) in orofacial clefting. Patients present with heterozygous mutations in nearly any region of this protein. Knock-down of *Specc1L* in cultured cells show deficits in the organization of the actin cytoskeleton and adherens junction complexes. Homozygous-null mice are lethal at E9.5, revealing arrest of cranial neural crest cells within the neural folds, that is, they do not undergo normal EMT and subsequent migration. Staining of adherens junction proteins showed that these junctions are more abundant in the mutant neural folds. Hypomorphic alleles of *Specc1L* were generated. These mice survive to birth and show blebbing and edema in the palatal shelves, but no clefting. Compound heterozygotes, on the other hand, show loss of palatal shelf elevation caused by oral adhesions between the epithelium and the tongue; this phenotype resolves by E15. Since this phenotype is similar to that reported for *Irf6* mutants, collaborative work with Dr. Brian Schutte's lab (Michigan State University) reveals that IRF6 is required for *Specc1L* expression in the palate in a ROCK1-dependent manner.

The last speaker in this session, **Loydie Jerome-Majewska** (McGill University), discussed her work on discovering the underlying genetic causes of Mandibulofacial Dysostosis with Microcephaly (MFDM). Combining forward genetic ENU screens in mouse and whole exome sequencing of patient DNA, she found mutations in *Eftud2* (elongation factor Tu GTP binding domain containing 2), that encodes a core spliceosome subunit whose GTPase activity is required to produce mature mRNAs. Homozygous nulls are preimplantation lethal, but embryonic phenotypes could be studied when the *Eftud2* deletion was driven only in the cranial neural crest by a *Wnt1-Cre* driver. Starting at E9.5, these embryos showed a reduced midbrain and became exencephalic by E14.5. The branchial arches were hypoplastic; neural crest cells migrated into branchial arch 1 normally but were reduced in the more posterior arches. Cartilage staining assays revealed abnormal morphology of the inner ear and Meckel's cartilage, whereas no abnormalities were detected in the limbs or vertebrae. Thus, a new model for understanding MFDM phenotypes is being developed.

7 | KEYNOTE ADDRESS: HUMAN BRAIN ORGANOIDS AS A MODEL FOR NEUROLOGICAL DISORDERS

The Keynote Address was given by **Alysson Muotri**, Professor of Pediatrics at the University of California in San Diego School of

Medicine. Dr. Muotri's laboratory focuses on modeling neurological disease using human pluripotent stem cells. His group has developed several innovative techniques for deriving human neurons and glia from iPSCs for basic research and drug-screening platforms. The goal of their research is to develop human brain organoids to directly address issues of human embryonic brain development, for which there is very little information. A major limitation to this approach has been that the neurons tend to be immature, the tissue is not vascularized and many cell types are missing. Innovative tinkering with the culture system based on our understanding of cortical development from animal models has led to major improvements including long-term viability (up to 2 years) of organoids that contain large numbers of cells, differentiated radial glial cells and neural progenitor cells that migrate into a structure that resembles the cortical plate. Single cell RNA-sequencing of these organoids reveal the presence of the three dominant cell types in the human cerebral cortex: glutamatergic neurons, GABAergic neurons and various glial cells. The transcriptomes of the organoids at various stages of culture indicate that neural-induced iPSCs resemble embryonic neural stem cells, 1-month organoids resemble fetal cortex and 3-month organoids resemble postnatal cortex. These organoids are being used to study several disease conditions. For example, how does the Zika virus cause microcephaly? Dr. Muotri's studies showed that infection occurs in the cortical layers of the organoids and selectively kills SOX2/PAX6-positive neural progenitor cells. By using this paradigm to screen anti-viral drugs, they found that Sofosbuvir blocks Zika replication and when given to a pregnant mouse can prevent the virus from crossing the placental barrier and infecting the embryos. In another study, Dr. Muotri's group asked whether these mini-brains can be used to understand neuronal networks. They showed that the neurons are voltage sensitive, express all the expected synaptic proteins and the network includes both excitatory and inhibitory neurons. When these cells are plated on multi-electrode arrays, bursts, spike events and synchrony events can be recorded over long periods, allowing one to monitor the development of neuronal networks. Interestingly, oscillations appear by 10–12 weeks in culture, and as they become more complex they resemble preterm infant EEGs. These cultures are now being used to determine what factors contribute to the emergence of human neural oscillations and to screen for drugs that might alleviate the oscillatory disruptions seen in Retts syndrome, autism, and schizophrenia. Future work also includes creating sensory-cortical region-specific complexes and using brain organoids to send signals to a machine interface. Because the development of brain organoids is similar to the trajectory of human brain development, the future application of this innovative technology to numerous questions of cortical communication in normal and disease conditions is exciting.

Members meeting

During the members meeting, Dr. Sally Moody (George Washington University) became the new President for 2019 and 2020. Dr. David Clouthier (University of Colorado, Anschutz Medical Campus) and Dr. Jean-Pierre Saint-Jeannet (New York University, College of Dentistry) were elected as the new Vice-President and Treasurer, respectively, for 2019 and 2020. Dr. Lisa Taneyhill (University of Maryland, College Park) will continue in her role as Secretary for 2019. The SCGDB also announced two "Excellence in Craniofacial Research" awards to commence in 2019. These awards are designed to recognize the research accomplishments of senior career and mid-career members of SCGDB and will be sponsored by *Developmental Dynamics* for 5 years.

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CONFLICTS OF INTEREST

None of the authors has a conflict of interest to declare.

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