Published in final edited form as:

Am J Med Genet C Semin Med Genet. 2013 November ; 163(4): 213–217. doi:10.1002/ajmg.c.31383.

Perspectives and Challenges in Advancing Research Into Craniofacial Anomalies

TIMOTHY C. COX*, DANIELA V. LUQUETTI, and MICHAEL L. CUNNINGHAM

Abstract

Development of the craniofacial region is a remarkably complex and tightly orchestrated process. It is therefore not surprising that genetic and environmental insults frequently result in craniofacial anomalies. Nonetheless, our knowledge of their etiology and pathogenesis is still scarce, limiting our efforts at prevention. Furthermore, few standardized protocols have been developed to guide clinical and surgical interventions. In this Issue of the Seminars, reviews on the most recent research advances on craniofacial conditions, from genomics and epigenetics to ontology and medical care are discussed with emphasis on the most common anomalies of the craniofacial region: orofacial clefts, craniosynostosis, craniofacial microsomia, facial dysostosis, Robin sequence, jaw and dentition anomalies, and anterior neural tube defects. Phenotypic variability and the importance of detailed characterization using standardized terminology to better distinguish between phenotypes, new technologies (and their limitations) for genetic diagnosis, and the use of mouse models to study these conditions in both their complex phenotypic and genetic aspects are highlighted.

INTRODUCTION

Why Craniofacial?

Craniofacial form is inextricably linked to our societal structure, essentially playing a major role in defining who we are. Not only does it provide most of the major senses that determine our ability to efficiently interact with our surroundings and the natural route by which we obtain nutrition to sustain life, but it also serves as the principal basis of our recognition of self and family (i.e., familiar and heritable features). To serve these roles, the overall structure of the craniofacial region is necessarily complicated—from the exquisite morphogenetic events that coordinate its assembly to the complexity of tissues and cell types that enable all its functions. It is not surprising then that it is also the single most impacted structure during development, whether as a result of genetic or environmental insult or a combination of the two.

The unfortunate downside to being a centerpiece of societal structure is the different treatment of, or inability to accept into “society,” those with atypical craniofacial form. While much of our healthcare is devoted to fixing the functional deficits of such birth defects, there is also a need to “normalize” an individual’s appearance so they can more easily integrate into society. However, our goal should not only be to do a better job at diagnosing or correcting craniofacial dysmorphologies, but should rather also be focused on mitigation and/or prevention. The latter goal is especially true if we are to make significant

inroads into alleviating the global burden of these conditions not just in Western societies, which already have access to quality healthcare, but to those societies that are less fortunate.

As health care professionals, there is a responsibility to keep abreast of the latest research advances and continually review practices to ensure the most optimal outcomes for patients. From experiences in our own Craniofacial Center, a coordinated multidisciplinary approach is essential for optimal care of the patient with a craniofacial anomaly. However, regular multidisciplinary discourse involving all health care specialties as well as researchers (epidemiologists, geneticists, developmental biologists, and epigeneticists to name a few) is required if significant advances are going to be made in understanding not only the causes of craniofacial anomalies but also the most effective evidence-based interventional and treatment strategies.

A Vision for the Future

“Why understand the genetic basis of craniofacial birth defects? What are you hoping to achieve with such an understanding? It is not as if you’ll ever be able to correct the genes in the developing human embryo. Plus, can’t all craniofacial malformations be surgically corrected anyway?” These are the types of questions and comments many of us used to hear frequently 20, and even 10, years ago both from grant reviewers and lay people who ventured unwittingly into conversation about what we did for a living. It is pleasing, not just as grant writers, that we no longer hear these comments. So what has changed?

First and foremost have been the tremendous advances in genetic technologies over the past decade and specifically in the way we analyze genomes—from arrayCGH, SNP arrays and, more recently, massively parallel (or deep) sequencing methods such as exome sequencing. These methodologies have proven exceptionally useful and powerful for family studies where there is a clear heritable phenotype and in studies with parent-case trios in instances where there is a robust and reproducible phenotype. Consequently, these technologies have raised so much hope for patients and families, in as much as a genetic diagnosis can bring with it some degree of certainty, closure, and understanding whilst also ascribing true recurrence risks. But even though these approaches have quickly become standard weapons in the geneticist’s arsenal, there are still numerous limitations, both technical and practical, as outlined in the review by Kriti Khan-delwal and colleagues. These limitations are particularly evident when dealing with common craniofacial disorders such as orofacial clefts (see review by Elizabeth Leslie and Mary Marazita) and craniofacial microsomia (review by Carrie Heike et al.) where phenotypic diversity, that is: variable expressivity and incomplete penetrance, is a major confounding factor.

The other major advance that has influenced perception is arguably in the field of epigenetics, and most significantly that involving the maternal–fetal interface, that is, hormonal, teratogenic, and nutritional impact on fetal disease susceptibility and long-term health and well-being. While gene–environment interactions have long been acknowledged as important, our ability to quantify specific effects both at the genome level and now at the phenotypic level has unveiled enormous possibilities. In this Issue, the extensive review by Claudia Kappen on anterior neural tube defects elegantly highlights the power of both epidemiological studies and particularly animal model investigations in teasing apart the important interrelationship between diet and genotype. A similar convergence of epidemiological and biochemical evidence is also raising hope for interventional and/or preventative therapeutic possibilities in other craniofacial conditions, such as the craniosynostosis and midface deficiencies, although much work is still to be done. In all cases, inroads can only be made with more detailed and quantitative phenotyping, an understanding of the functional impact of genetic variants, and a much better appreciation of the full spectrum of clinical variability in all craniofacial conditions. With this in mind, the

face and the craniofacial skeleton have considerable advantages over other organ systems in that many tools are already available for quantitative 2D and 3D imaging, including computed tomography (CT), magnetic resonance imaging (MRI) and 3D photogrammetry, and in at least the larger craniofacial centers are a routine part of treatment planning and clinical care. The key to rapid progress in this area however will be the standardization of protocols used by clinicians in different centers. The review by Carrie Heike and colleagues expounds the benefits of such an approach for both understanding and managing craniofacial microsomia, providing a framework on which we can measure the effects of ever changing treatment strategies and serving as an excellent model for other conditions.

DEALING WITH THE CHALLENGES POSED BY PHENOTYPIC VARIABILITY

As many of the articles in this Issue illustrate, even with the extraordinary power to detect causative mutations that is afforded by different genetic technologies, we still cannot predict who will present with a particular craniofacial condition even when carrying an identified causative mutation. And for many conditions, there is even variability in surgical outcomes for children with the same clinical diagnosis that is not related to the surgeons expertise. Understanding the major factors that contribute to these issues endure as some of the biggest challenges in craniofacial medicine.

Phenotypic variability can take the form of variable expressivity (the variation in the degree to which tissues are affected) and incomplete penetrance (the absolute variation of severity from “full blown” to the apparent absence of clinical signs). So what factors contribute to this phenotypic variability? In the past decade or so, emphasis was on ‘other’ genetic variation within an individual’s genome, largely because the technology to address this issue was much further advanced than for other areas. However, in more recent times there has been a significant shift to focus on the role of epigenetics—in the broadest sense, being defined as factors not determined *per se* by an individual’s DNA sequence but that nevertheless influence gene expression and activity. In humans, the discordance of phenotypes in identical twins is the classic indicator of the relative contribution of genetic variation and epigenetic factors.

Animal models, particularly mouse models, also provide many spectacular examples of phenotypic variation due to the influence of epigenetic factors. Because most investigators generate and maintain their models of craniofacial dysmorphology on inbred (i.e., pure) genetic backgrounds, any inter-individual variation in phenotype can be considered a result of epigenetic influences. Just as striking can be the differences in phenotypic presentation (including variability) when specific mutations are introduced or maintained on different inbred genetic backgrounds in this species. Classic examples of this are the *Tcof1* mutations (which in humans cause Treacher Collins syndrome) and the epistatic *clf* (*clf1* and *clf2*) alleles that confer cleft susceptibility. In fact, it is expected that most mutations would show phenotypic diversity if placed on different inbred or even outbred backgrounds, thus extending the power of these mouse models to also map genetic modifiers and dissect gene–environment interactions. However, as with clinical studies, the power of such approaches is currently limited by the ability to quantify changes in gene expression and phenotypes with sufficient resolution and sensitivity (and of course funding!). For gene expression, microarrays still offer a relatively cheap means of detecting more robust changes, whereas deep sequencing-based approaches such as RNAseq and even ChIPseq (and variations thereof) are promising to provide unprecedented insight into changes in expression, tissue-specific patterns of mRNA splicing and epigenetic mechanisms of gene regulation. And for phenotypes, particularly in animal models, significant advances are being made using technologies such as micro-CT, micro-MRI, and various new laser-based microscopic techniques.

Phenotyping and Subphenotyping

In this Issue of the Seminars, we not only wanted to provide updates on the state-of-the-field for the most common, and arguably the more significant, craniofacial conditions that present in clinic (see reviews by Elizabeth Leslie and Mary Marazita, Carrie Heike et al., Tony Roscioli et al., and Claudia Kappen), but also to highlight the advantages of combining animal model investigations with clinical studies. To this end, a number of reviews in this Issue are co-authored by developmental biologists and clinicians who are either already working together or have come together for this Issue to provide a unique perspective on specific craniofacial conditions (see reviews by David Clouthier et al., Paul Trainor and Brian Andrews, Peter Farlie et al., and Ophir Klein et al.).

It is noteworthy that a number of these articles cross into topics covered in other reviews in the Issue because of phenotypic similarity between these groups of craniofacial disorders. In fact in some cases, these other disorders were originally considered as differential diagnoses. For example, in the review on craniofacial microsomia, both the facial dysostoses, auriculocondylar syndrome, and Pierre Robin sequence are discussed, and vice versa for the reviews on each of these three conditions. While these examples highlight the benefits of careful phenotypic descriptions and recognition of facial gestalts, they also extol the virtues of obtaining a definitive genetic diagnosis. Using the auriculocondylar syndrome as a case in point, retrospective assessment of individuals harboring either *PLCB4* or *GNAI3* mutations suggests there may be features distinguishing each of the genetic subtypes (see review by David Clouthier et al.). The same applies to the facial dysostoses such as Mandibulofacial Dysostosis with Microcephaly (caused by mutations in *EFTUD2*) and Nager syndrome (caused by mutations in *SF3B4*) (see review by Paul Trainor & Brian Andrews). While these and the other facial dysostoses show many similarities because the causative genes are believed to function in the same biological processes, the individual disorders can be distinguished in most cases. However, this ability relies on an appreciation of the full phenotypic spectrum associated with each gene, and in some cases this may require a shift to quantitative phenotyping, which by its nature requires normative data for comparison.

While the notion of detailed phenotyping is not new, there is now certainly a new appreciation for the level of precision that is required to distinguish closely related phenotypes, and in many cases this may only be possible through quantitative measures. Such detailed assessment is relatively simple if conducted retrospectively with genetic data in hand, but can, as a result, unwittingly lead to bias. Therefore, careful subphenotyping ideally should be performed first to provide the recommendation for targeted gene analysis rather than the other way around. This does not only make sense from a research perspective but also from a cost perspective. The review by Tony Roscioli and colleagues on their experiences in Australia with a national diagnostic service for the craniosynostoses provides a simple example of a cost-effective service to gather genetic data on a large volume of phenotypically well-defined patients that would otherwise not be possible through individual clinical activities. There are many other opportunities where detailed and even quantitative phenotyping could easily have an impact. For example, it is somewhat surprising that today the vast majority of dysmorphic patients are still screened for “del22q11.2” even if the typical facial gestalt and cardiac defects of this well-characterized syndrome are absent. Does this reflect inexperience of the referring clinicians or a “catch all” in case it is an atypical presentation? There are two things to consider here: (1) cost and (2) interpretability. If the result came back positive for the typical 22q11.2 deletion, can it really be interpreted as causing the variant phenotype or is it a simple case of non-penetrance, with some other lesion being responsible? If it really isn't interpretable, then the cost is not justifiable. Now, with the costs of the newer genome technologies, such as exome sequencing, become more affordable, such approaches will no doubt be added to the list of potentially un-interpretable

exploratory tests “performed” on poorly phenotyped patients. Yet it is hoped that in the future these new technologies can be paired with both highly detailed phenotyping protocols as well as “intelligent” data and bioinformatic search algorithms to instead generate testable research hypotheses (i.e., using knowledge of gene expression in model organisms, genetic pathways, or cell and developmental processes either for targeted locus sequencing or for prioritizing analyses of genomic scale data). This topic is addressed in the review by Jim Brinkley and colleagues.

For more genetically complex craniofacial conditions, such as orofacial clefting, the need for careful, detailed subphenotyping is already well recognized and nicely outlined by Elizabeth Leslie and Mary Marazita in their review. As part of this, and also emphasized by Ophir Klein and colleagues, is the importance of noting associated features, especially those derived from embryo-logically related tissues or processes. For example, although noted decades ago, a recent flurry of articles has seen the re-investigation of the co-presentation of tooth defects in individuals with orofacial clefts. In each of these studies, a significantly increased frequency of an array of dental anomalies, including missing and supernumery teeth, malformed teeth, as well as defects in mineralization, has been found, particularly in those with cleft lip/palate. These data support the notion of a common etiology. Consistent with this is the fact that most major cleft lip/palate genes are expressed in the embryonic oral epithelia (not the neural crest derived mesenchyme, as commonly assumed), which also plays a major role in both induction and mineralization of teeth. Animal models will now be critical for identifying the common molecular and developmental mechanisms, which may ultimately provide new clues to better manage the dental issues faced by this group of patients.

ANIMAL MODELS—APPRECIATING THE BENEFITS AND LIMITATIONS

As emphasized above and elegantly discussed in many of the reviews in this Issue of Seminars, animal models—particularly the mouse—continue to play a major role in helping us understand the molecular and developmental basis of craniofacial disorders. In fact, with the massive international expansion in mutant mouse resources, through numerous federally and internationally funded gene knockout, chemical mutagenesis and spontaneous mutant surveillance programs, this contribution is expected to exponentially grow. However, other model systems, such as the zebrafish (as outlined by Ophir Klein et al.) and the chick, are also being increasingly used in craniofacial research as genetic resources and techniques for genetic manipulation in these species are rapidly being developed. Although the broader utility and relevance of these alternate model systems may seem questionable on the surface, they each have distinct advantages and limitations that must be considered when contemplating them as a research model for craniofacial phenotypes. For example, as Klein et al. point out, zebrafish offer many advantages for dissecting genetic pathways that are typically conserved in vertebrate craniofacial development. They can justifiably be used as models for early mandibular development and branchial arch patterning as well as even studies on calvarial ossification and suture biology. However, their suitability for studies of midfacial development is uncertain because of the questionable homology of many of the zebrafish midfacial cartilages to the mammalian facial skeleton. Although the same might once have been considered for teeth, as Klein et al. explain, zebrafish still have ancestral palatal teeth that appear to form and mineralize using many of the same molecular pathways as in mammalian dentition.

THE IMPORTANCE OF TERMINOLOGY

Aside from the difficulty of obtaining funding, two simple things provide the most source of frustration from a research perspective: a lack of detail in phenotypic descriptions (as

discussed above), and the poor or inappropriate use of terminology. In fact, these issues pertain equally to clinical studies and animal model investigations. In part they can reflect an availability of necessary resources (e.g., insufficient tools for assessment, or lack of time or expertise of the investigator). However, they also can result from a lack of appreciation for the need of detail or accuracy, or alternatively the acceptance and propagation in the literature of ill-defined or misused terms. An example of the latter that remarkably still occasionally appears in the current literature is the inappropriate use of the term “cleft lip with or without cleft palate” (CL/P) to describe individuals with only a secondary palatal cleft. Many clinical investigators have previously acknowledged or recognized similar issues, which led to the coordinated effort and publication, in this Journal, of the Elements of Morphology [Allanson et al., 2009], a large set of well-defined (and agreed upon) clinical terms to describe non-canonical or atypical craniofacial morphology [Carey et al., 2012]. This was an important step in the right direction.

In this Issue we therefore also felt it prudent to include an update on recent efforts, facilitated by the National Institute of Dental and Craniofacial Research’s FaceBase Initiative, to formalize and extend an ontological resource that underpins research into craniofacial development and dysmorphology (see review by Jim Brinkley and colleagues). This large project, The Ontology of Craniofacial Development and Dysmorphology (OCDM), is built around the Foundational Model of Anatomy (FMA), which is the most widely utilized biomedical ontology. While the goals of the OCDM are not to force researchers to use specific terminology, its purpose is to define terminology (in humans and mice) and provide their anatomical relationships both to other terminology, to related or derivative anatomical structures (even across species), as well as to originating embryologic anatomy—both in the context of canonical anatomy and dysmorphology. With the need for more detailed phenotyping and the technology capable of better discerning or distinguishing similar phenotypes, we feel it is important that all craniofacial researchers are aware of these efforts and the resources they will ultimately provide.

CONCLUDING REMARKS

The ability to understand the causes and improve the treatment of craniofacial disorders does not just come with a genetic diagnosis but requires considerably more detailed information on pathways and developmental processes that can only come from research on appropriate animal models. But in the clinic, there also remain many challenges. To improve diagnoses and, as a consequence, prognoses we need to utilize more precise and detailed methods for describing phenotypes, especially where marked variability is presumed or anticipated. Understanding both the genetic and epigenetic contributions to phenotypic variability will not only help interpret how these factors influence clinical outcomes and treatment practices, but ultimately will also provide insight into the exciting realm of interventional or preventative therapeutic strategies.

References

- Allanson JE, Cuniff C, Hoyne HE, McGaughan J, Muenke M, Neri G. Elements of morphology: Standard terminology for the head and face. *Am J Med Genet A*. 2009; 149A:6–28. [PubMed: 19125436]
- Carey JC, Allanson JE, Hennekam RC, Biesecker LG. Standard terminology for phenotypic variations: The elements of morphology project, its current progress, and future directions. *Hum Mutat*. 2012; 33:781–786.

Biographies

Timothy Cox, Ph.D. is Professor and Laurel Endowed Chair in Pediatric Craniofacial Research in the University of Washington's Department of Pediatrics (Division of Craniofacial Medicine) and the Center for Developmental Biology and Regenerative Medicine at Seattle Children's Research Institute. His research employs animal models to understand the genetic and epigenetic contributions to craniofacial development and dysmorphology.

Daniela Luquetti, M.D., Ph.D. is a medical geneticist and epidemiologist in the Craniofacial Center at Seattle Children's Hospital and the Division of Craniofacial Medicine, Department of Pediatrics, University of Washington. Her research includes studying potential genetic and non-genetic causes of birth defects.

Michael Cunningham, M.D., Ph.D. is the Jean Renny Professor of Craniofacial Medicine in the University of Washington Department of Pediatrics and Medical Director of the Seattle Children's Craniofacial Center. For over 20 years Dr. Cunningham's career has focused on the care of children with craniofacial conditions as well as basic and translational research to identify causes and treatments.