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Facing up to the Challenges of Advancing Craniofacial Research

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Abstract

Craniofacial anomalies are among the most common human birth defects and have considerable functional, aesthetic, and social consequences. The early developmental origin as well as the anatomical complexity of the head and face render these tissues prone to genetic and environmental insult. The establishment of craniofacial clinics offering comprehensive care for craniofacial patients at a single site together with international research networks focused on the origins and treatment of craniofacial disorders has led to tremendous advances in our understanding of the etiology and pathogenesis of congenital craniofacial anomalies. However, the genetic, environmental, and developmental sources of many craniofacial disorders remain unknown. To overcome this problem and further advance craniofacial research, we must recognize current challenges in the field and establish priority areas for study. We still need (i) a deeper understanding of variation during normal development and within the context of any disorder, (ii) improved genotyping and phenotyping and understanding of the impact of epigenetics, (iii) continued development of animal models and functional analyses of genes and variants, and (iv) integration of patient derived cells and tissues together with 3D printing and quantitative assessment of surgical outcomes for improved practice. Only with fundamental advances in each of these areas will we be able to meet the challenge of translating potential therapeutic and preventative approaches into clinical solutions and reduce the financial and emotional burden of craniofacial anomalies.

Keywords

craniofacial anomaly; genetics; development

INTRODUCTION

The craniofacial complex comprises an intricate assembly of specialized tissues and organs including the majority of the primary sense organs, the central and peripheral nervous

systems, and musculoskeletal components of the head and neck. Because of its unique evolutionary history, the craniofacial skeletal system is composed of bones that derive from two separate sources (neural crest and mesoderm) and is a composite of endoskeleton forming primarily endochondrally, and exoskeleton (dermatocranium) forming intramembranously [Hall, 2014].

Anatomically and developmentally, the head is the most complex region of the human body and prone to genetic and environmental insult. Of the 1% of all live births that exhibit some form of minor or major anomaly, approximately one-third affect the head and face [Gorlin et al., 1990]. Currently, more than 700 distinct craniofacial syndromes have been described, and orofacial clefts (~1 in 1,000 live births; [Schutte and Murray, 1999]) and craniosynostosis (~1 in 2,500 live births [Johnson and Wilkie, 2011]) represent two of the most common congenital craniofacial defects.

Craniofacial anomalies have serious functional, aesthetic, emotional, and social consequences that require extensive clinical and surgical management. A multidisciplinary team involving geneticists, neuro-, plastic and oral surgeons, otolaryngologists, orthodontists, audiologists, psychologists, speech and language pathologists, nurses, and social workers is required to adequately diagnose, treat, and ameliorate individual conditions. Healthcare for patients with craniofacial anomalies thus comes at a great emotional and financial cost over many years, with the results often variable, and rarely fully corrective.

INTERNATIONAL NETWORK

Though there was a movement in the 1980s to establish craniofacial clinics offering comprehensive care for craniofacial patients at a single site [Kaye and Rollnick, 1985], up until about 15 years ago, most of the research into, and treatment of, craniofacial disorders, was conducted independently with little evidence of a coherent global strategy. In 2000, with the financial support of the United States National Institute of Dental and Craniofacial Research, the World Health Organization initiated the International Collaborative Research on Craniofacial Anomalies project. This effort was designed to establish an international network focused on research in craniofacial anomalies under priority areas including the following: the genetic basis of craniofacial anomalies, hereditary and environmental interactions, and optimal treatment and prevention of craniofacial anomalies.

ADVANCES INFORM FUTURE DIRECTIONS

The results have been remarkable. During the past 15 years, the integration of human and animal genetics with experimental embryology, cell biology, and biochemistry has dramatically improved our fundamental understanding of the normal processes of craniofacial development and provided novel insights into the etiology and pathogenesis of many craniofacial conditions. Unfortunately, despite this tremendous progress, the precise mechanisms of many of the most common and numerous craniofacial disorders remain unknown. This lack of knowledge means that the challenge of translating potential therapeutic and preventative approaches into the clinic remains unanswered. Therefore, progress in the following priority areas is still required. First, the complete spectrum of

phenotypic variation during normal craniofacial development needs to be quantitatively detailed, most likely by the combined processes of research on human cases coupled with computational modeling of potential phenotypes. Second, earlier detection and more accurate phenotype–genotype correlations need to be obtained. Third, more statistically based standardized protocols that provide guidelines for when and how to treat specific anomalies that result in best case predictable outcomes need to be generated. Finally, tissue-engineering approaches incorporating patient-derived tissue or induced pluripotent stem cells (iPSCs) together with 3D printing need to be integrated to enable customized reconstruction and a more natural healing process.

There have been major technological advances in the way we obtain and analyze genomes, including whole exome and genome sequencing, and comparative genome hybridization (CGH) and single-nucleotide polymorphism (SNP) arrays.

These approaches are extremely powerful when applied to heritable conditions as in the case of parent–case trios with a consistent and reproducible phenotype [Cox et al., 2013]. However, in many cases, the genomic technologies have advanced faster than our ability to thoughtfully deal with the avalanche of data. The etiology of relatively few complex craniofacial disease traits have been revealed using the principles of Mendelian inheritance established during the early half of the twentieth century, and even our understanding of normal variation of craniofacial phenotypes is deficient. Currently, the major non-genetic factors that are thought to interact with and contribute to phenotypic variation are environmental agents and epigenetic factors. The diversity of disease phenotypes is often attributed to variable expressivity and incomplete penetrance, terms that describe the range of traits that can occur in different people with the same genetic condition and the proportion of people that carry a genetic trait that exhibit traits of the disorder, respectively.

These terms provide statistical summaries of the relationship between defined genetic variants and observable phenotypes, but do not provide an explanation for the observed variation.

Variation in phenotypes is likely caused by a combination of genetic, environmental, and lifestyle factors, many of which may not yet be identified. The discordance of phenotypes in identical twins, a classic indicator of the relative influence of genetic variation versus epigenetic factors, can provide important insights into the etiology and pathogenesis of craniofacial malformations. However, twins are a fairly limited resource that cannot drive fundamental insights into the mechanisms underpinning the tremendous variety of craniofacial anomalies. The pathogenesis of the most common forms of craniofacial anomalies, the “non-syndromic” conditions, remains particularly challenging because they most likely arise from a combination of complex polygenic interactions with environmental influences. For these, the challenge is to go beyond the enumeration of potential contributing factors (genetic, epigenetic, and environment) to methods and models that begin to analytically account for the integration of these factors in the production of phenotypes.

Careful phenotyping has always been a hallmark of the study of craniofacial anomalies, but the persistent finding of unexplained variation points to an urgent need for more detailed,

quantitative phenotyping to truly appreciate the complete spectrum of clinical variation across all craniofacial conditions. Developmental biology has helped us recognize the persistence and extent of variation, even among genetically identical littermates in animal models. Using statistical and morphometric methods of analysis coupled with ideas from population and quantitative genetics, researchers have started to explore the “craniofacial phenotypic landscape” associated with specific genes or mutations within those genes [Heuzé et al., 2014b; Young et al., 2010, 2014]. Quantitative measures of even subtle phenotypes in animal models provide information that was not previously available. Preliminary results suggest that some conditions can be described by a spectrum of severity (e.g., Heuzé [2014b]), while others fit the classical idea of a threshold trait. Again, the developmental, genetic and environmental contributions to these diverse phenotypes are unknown. But just as hypothesis-generating and predictive approaches in biomedicine have become possible with the decreased cost of increasingly powerful genomic assays [Biesecker, 2013], the rapid advance of new imaging techniques, 3D morphometric analyses, and computational modeling releases scientists from testing phenotype-specific hypotheses and allows the exploration of potential or predictive morphologies facilitating insights into variability and constraints on evolution and development.

Given the diverse sources and the wide range of phenotypic variation, it is not surprising that there are few standardized protocols for guiding clinical and surgical intervention. Additionally, there has been little rigorous quantitative assessment of the outcome of specific procedures from a temporal, functional, and aesthetic perspective. Contributing to this state of affairs is that beyond a lack of understanding of sources of variability in craniofacial anomalies, we lack a thorough quantitative understanding of the real and potential range of variation during normal craniofacial development. Not surprisingly, many craniofacial syndromes present with similar and, in many cases, overlapping features. This is particularly true with respect to facial dysostosis, which encompasses the mandibulofacial dysostosis and acrofacial dysostosis conditions [Trainor and Andrews, 2013]. A similar phenomenon is observed in relation to craniosynostosis [Johnson and Wilkie, 2011; Jezela-Stanek and Krajewska-Walasek, 2013] and holoprosencephaly [Petryk et al., 2015]. These observations caution the use and re-use of terms which describe common craniofacial disease traits (e.g., midfacial hypoplasia) that foster the idea that similar localized phenotypes are shared by many syndromes. In some cases, these disease traits may share only superficial, generalized anatomical similarities and their etiology, detailed anatomy, and developmental history may vary, necessitating different treatment regimes.

Many of the major gene families (e.g., FGF, SHH, BMP, and Wnt) have been implicated in certain craniofacial conditions and some are known to cooperate in the coordination of major biological processes. Identification of susceptibility genes and enumeration of the most promising genetic variants is not sufficient for understanding the biological mechanisms that underlie normal morphogenesis, or by extension, dysmorphogenesis. Experimental findings repeatedly suggest complex interactions of new genetic variants and gene families with at least partially known functional properties during development. For example, recent genomic research into the genetic basis of non-syndromic craniosynostosis (approximately 85% of all cases of craniosynostosis) points to newly identified genes that operate in signaling pathways previously found in syndromic cases of craniosynostosis

[Heuzé et al., 2014b; Justice et al., 2012] that are vital to normal development. These findings suggest a tight coupling of many craniofacial developmental processes and call for new methods for understanding the genetic networks that supervise development.

Accurate detailed phenotyping and subphenotyping are essential to make sense of whole genome and exome sequencing for any patient cohort and represents an additional, vital step toward understanding biological pathways contributing to common anomalies. Patterns of anomalies quantified by morphometric approaches associated with knowledge of the genetic risk burden, and functional studies to determine functional consequences of the most promising genetic mutations, rely on an appreciation of the full phenotypic spectrum of craniofacial disorders, and this depends upon normative data for comparison. If genetic tests return results but the patients have been poorly or inadequately phenotyped, assigning genetic causality becomes a costly and fruitless exercise [Brinker and O'Connor, 2013; Roscioli et al., 2013]. Accurate phenotyping and subphenotyping become even more important with the very real possibility of all new born babies eventually having a whole genome sequence as part of their permanent medical and health records.

Coinciding with the continual improvement in medical and surgical care in the management of craniofacial syndromes, early in-utero detection and phenotyping have the potential to facilitate intervention and minimize the manifestation of anomalies prior to birth. Ultimately the goal for managing any craniofacial anomaly continues to be prevention, but the development of therapeutics for minimizing or preventing craniofacial anomalies requires an understanding of the precise etiology and pathogenesis of individual malformation syndromes. Central to this is a better appreciation of the distinct signals, switches, and mechanism that regulate normal development of the head and neck [Trainor, 2013]. Achieving these goals involves a deeper understanding of the functional impact of genetic variants, both individually and in combination. For this reason, animal models will continue to play a major role in helping define the mechanisms and processes governing normal craniofacial development, and the etiology and pathogenesis of craniofacial anomalies. Importantly, each animal model species has its own unique evolutionary history that conveys advantages and disadvantages for specific research protocols and purposes and can reveal differences that are critical to a deeper understanding of the conservation and diversity of mechanisms across species.

Finally, craniofacial surgery presents complex anatomical challenges to surgeons. Advances in tissue engineering and regenerative medicine have begun to provide new therapeutic options for craniofacial repair. However, one of the major challenges craniofacial researchers face is in translating basic bench discoveries into clinically relevant bedside treatments. Although complex tissue structures can be generated in a dish, they need to meet the criteria of being sustainable, functional, biomechanically sound, and aesthetically acceptable to be fully utilized in craniofacial reconstruction. Further advances in tissue-engineered reconstruction need multidisciplinary research to create complex tissue structures and make satisfactory outcomes clinically achievable for most patients [Schantz et al., 2012].

The integration of patient-derived tissue and induced pluripotent stem cells with rapid advances in 3D printing of biological tissues and materials holds great promise for customizing craniofacial implants for repair and reconstruction with the added benefit of invoking natural healing responses for more successful and satisfying long-term results.

CONCLUSIONS

The face is regarded as the organ of emotion, conveying a wealth of information that guides our social interactions and our identity of self.

Unfortunately, congenital craniofacial anomalies are among the most prevalent of birth defects. This significantly impacts infant mortality and dramatically affects national healthcare budgets. Although we have witnessed tremendous advances in our understanding of craniofacial development and disease, the etiology and pathogenesis of numerous craniofacial disorders remain a mystery. To address this shortcoming, we need (i) a deeper quantitative appreciation of the spectrum of variation during normal craniofacial development and with respect to each individual craniofacial condition, (ii) earlier detection and more precise phenotyping of individual syndromes (and individuals!) and conditions to facilitate more accurate causative genetic correlations, (iii) continued development of animal models for exploration of phenotypic variation and functional testing of genes and their variants during normal craniofacial development and in the pathogenesis of disease, (iv) an increased awareness of the gene-environment interactions and the impact of epigenetics, (v) integration of patient derived cells and tissues together with advancing tissue-engineering tools such as 3D printing, (vi) more rigorous quantitative temporal, functional and aesthetic assessments of surgical procedures and their outcomes for better diagnostic and prognostic predictions of outcome, (vii) design of therapeutic approaches for prevention of congenital craniofacial defects, and (viii) a deeper understanding of the “life history” of craniofacial conditions with an eye toward anticipating major events in pre- and post-natal development that could be targeted by therapeutic means. Achieving these objectives requires a comprehensive and thorough understanding of the normal events controlling craniofacial development during embryogenesis.

One of the primary goals of the Society for Craniofacial Genetics and Developmental Biology is to bring together basic scientists, clinical geneticists, clinicians, and surgeons to integrate basic and clinical research and treatment with the ultimate goals of improving the clinical care of patients with craniofacial malformations, and educating and communicating new ideas to the scientific community and to the public. With continued advances and increased communication among researchers and care givers, the future looks bright for managing, treating, and preventing congenital craniofacial disorders.

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