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Hunting for genes that shape human faces: initial successes and challenges for the future

S.M. Weinberg^{1,2,3,*}, J. Roosenboom¹, J.R. Shaffer^{1,2}, M.D. Shriver⁴, J. Wysocka^{5,6}, and P. Claes^{7,8,9}

¹Center for Craniofacial and Dental Genetics, Department of Oral Biology, University of Pittsburgh, Pittsburgh, PA, USA

²Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA, USA

³Department of Anthropology, University of Pittsburgh, Pittsburgh, PA, USA

⁴Department of Anthropology, Pennsylvania State University, State College, PA, USA

⁵Department of Chemical and Systems Biology, Stanford University School of Medicine, Stanford, CA, USA

⁶Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA, USA

⁷Department of Electrical Engineering, ESAT/PSI, KU Leuven, Leuven, Belgium

⁸Medical Imaging Research Center, MIRC, UZ Leuven, Leuven, Belgium

⁹Murdoch Childrens Research Institute, Melbourne, Victoria, Australia

Abstract

There is ample evidence from heritability studies, genetic syndromes, and experimental animal models that facial morphology is strongly influenced by genes. In this brief review, we present an up to date overview of the efforts to identify genes associated with the size and shape of human facial features. We discuss recent methodological advances that have led to breakthroughs, but also the multitude of challenges facing the field. We offer perspective on possible applications of this line of research, particularly in the context of the precision genomics movement.

Keywords

GWAS; face shape; genotype-phenotype correlation; heritability; normal-range variation; complex genetic traits

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Human faces exhibit a high degree of morphological variation.¹ This variability arises, in part, from the intrinsic complexity of facial morphogenesis and the multitude of forces and factors that impact facial morphology over the lifespan. Genetics is clearly one of these factors, playing a critical role in defining the size and shape of our facial features. Verifying

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*Corresponding Author: Seth M. Weinberg, Center for Craniofacial and Dental Genetics, Department of Oral Biology, University of Pittsburgh, 100 Technology Drive, Suite 500, Pittsburgh, PA 15219, smwst46@pitt.edu.

this claim often requires little more than examining faces within one's own family or marveling at the uncanny similarity of identical twins. Large-scale expression studies have shown that a large number of genes are involved in the formation of the face.² Many syndromes with specific patterns of craniofacial dysmorphology result from single gene mutations³ and altering genes in animal models can result in a wide variety of craniofacial outcomes.⁴ However, the genetic factors that influence common facial traits in humans – the type of normal-range variation which gives each of us our unique facial appearance – is still poorly understood. An improved understanding of the genetic architecture of normal facial variation can not only provide insights into the biological mechanisms that control facial development, but can offer clues to help decipher the causes of anomalies involving the same facial structures. In the long term, our knowledge about the relationship between genotype and facial phenotype may inform personalized treatment planning in fields like orthodontics.⁵ There have now been a number of studies attempting to identify genetic variants associated with normal-range variation in facial morphology. In this brief review, we discuss the genetic evidence accumulated to date, some recent methodological advances, and some of the major challenges the field is facing.

Heritability of facial traits

Heritability is a measure of the proportion of phenotypic variation for a given trait in a given population that is accounted for by genetic factors. There have been many formal heritability studies of facial morphology. In principle, such studies can help point to which facial features are most and least likely to be influenced by genes. Several approaches have been used to study the heritability of facial features, including traditional family-based designs (twin or parent-offspring comparisons) and population-based methods.

Since the 1950s, numerous studies have attempted to estimate the heritability of simple anthropometric or cephalometric measurements of the craniofacial complex.^{6–8} Some more recent studies with access to large 3D facial datasets have employed more advanced morphometric methods in an effort to better quantify complex aspects of soft-tissue facial morphology.^{9,10} Collectively, these studies report a wide range of heritability estimates, even when the same measurement is considered, making generalizations difficult. However, virtually all of these studies report high heritability ($h^2 > 60\%$) for at least a subset of facial traits, with the majority of traits typically falling in the moderate heritability range. Moreover, studies focusing on soft-tissue morphology tend to report the highest heritability for aspects of nasal shape. This finding has been largely substantiated by genetic association studies (discussed below).

Measuring faces and mapping genes

Only relatively recently have scientists begun identifying the specific genes that impact normal-range human facial morphology. The earliest studies applied a candidate gene approach, whereby a set of polymorphic markers in and around specific genes are tested for a statistical relationship with one or more quantitative facial measures. The choice of genes is typically based on prior knowledge of biological relevance (e.g. expression in relevant tissues during development or causal for syndromes showing facial dysmorphology). Such

candidate studies have reported associations between well-known craniofacial genes such as *FGFR1*,¹¹ *IRF6*,¹² *LRP6*,¹³ and *GHR*¹⁴ and a variety of facial shape measures. Such studies have inherent limitations, because they require upfront guesswork to pick the right genes and variants for testing.

Genome-wide association studies (GWAS) avoid this limitation by simultaneously testing a dense panel of millions of variants spread across the entire genome, some located within genes but many in the vast stretches of DNA situated between genes. Because the precise location of every variant in the human genome is known, a statistical association with one of more variants provides evidence that a gene or regulatory element near that location is important. One downside of GWAS, however, is that the burden for statistical significance is very stringent due to the multiple test correction. To date, there have been nine GWAS focused on quantifiable aspects of human craniofacial morphology.^{15–23} The main characteristics and results of these studies are summarized in Table 1.

For the most part, each of these studies has identified a handful of associations. It is notable that the vast majority of the associated loci point to biologically plausible genes, which have been implicated in human craniofacial syndromes or malformations and/or show expression in developing craniofacial tissues. *PAX3* is a good example and was one of the first genes implicated in a facial GWAS.¹⁵ This gene is critical for the proper development of neural crest-derived structures in the face. While common variants in regulatory elements near *PAX3* are associated with normal-range upper nasal morphology in humans, damaging mutations in this gene are associated with Waardenburg syndrome (where this same anatomical region is dysmorphic). Moreover, *PAX3* is now one of several genes reported to show similar phenotypic associations across multiple independent studies.^{16,17,21} This type of independent confirmation is critical for distinguishing true signals from false positives. Collectively, these gene mapping efforts have been moderately successful at identifying genetic variants that influence human facial morphology.

Improved phenotyping, improved results

It is worth asking why prior GWAS studies have tended to identify so few loci. After all, GWAS of other polygenetic morphological traits, such height or BMI, have identified hundreds of independent genomic signals.²⁴ Moreover, large-scale expression studies in mice have revealed that thousands of genes are active in the developing face.² One simple reason is that facial GWAS have tended to involve modest sample sizes (typically under 5000 individuals), which results in low power to detect variants with small effects. With increased samples sizes, facial traits may indeed start to look polygenic. Genomic studies looking at non-quantitative facial features illustrate this point.^{25,26} For example, Shaffer et al.²⁶ recently identified 49 independent loci for earlobe attachment in a sample of over 70,000 individuals. It is probably a safe assumption that the genetic architecture underlying shape of the nose or chin is similarly polygenic. Combining datasets is the most obvious way around the sample size limitation, but inconsistent phenotyping and an inability to share the raw underlying data (often facial images) have been major impediments.

The phenotyping strategies used in prior GWAS have also been an important limiting factor. Typically, a set of facial measurements (often linear distances) are chosen by the instigators a priori, often measured on a set of 2D or 3D facial images, and then treated as univariate traits in a GWAS. One problem with this phenotyping approach is that there is no real biological justification for choosing one set of measurements over another. An additional problem is that such simple measurements cannot adequately capture complex shape, allowing only a crude modeling of gene effects. In a recent study published in *Nature Genetics*,²¹ a novel phenotyping approach was introduced that addresses these limitations by implementing a data-driven (unsupervised) approach to phenotyping based on machine-learning principles. By applying Procrustes-based methods that leverage the wealth of geometric information available in 3D facial surface images and exploiting the intrinsic multi-partite property of human faces, each individual's facial surface morphology was divided into a set of related but distinct modules (FIGURE 1). Starting with whole facial surfaces containing about 10,000 3D points in dense correspondence across a dataset, the multivariate correlations among all 10,000 points were computed and embedded in a spectral matrix. Spectral hierarchical clustering methods were then applied to discover groups of 3D points that show strong morphological correlations. This process was repeated successively until 63 modules, each representing a relatively distinct unit of facial morphology, are generated. A multivariate GWAS was then conducted on measures (principal components) that capture pertinent shape information for each 3D facial module. This highly efficient approach allows all aspects of facial morphology to be tested with a relatively low computational and statistical correction burden. Moreover, by nesting the modules within a hierarchical arrangement, genetic effects on facial morphology can be modeled simultaneously at multiple levels of scale, ensuring that genetic variants with highly localized effects are captured.

Despite a modest sample size of just over 2300 participants, this approach yielded 38 genome-wide significant loci involving 1932 common variants. A total of 1821 of these discovered variants (94.3%) at 15 loci were then fully replicated in an independent cohort. In addition, when implicated variants from prior facial GWAS were tested, almost all were replicated. The association signals were near many biologically relevant genes (e.g. *SOX9*, *TBX15*) and showed enrichment for biological processes critical for craniofacial development, including the regulation of cranial neural crest cell activity. The bulk of associated variants had phenotypic effects on nasal morphology (FIGURE 1), which accords with studies that show the highest heritability for nasal features. Moreover, the global-to-local phenotyping revealed genetic variants with distinctive phenotypic association patterns. For example, some variants impacted broad regions of the face represented by many modules, while others had very focal effects on morphology. The ability to map morphological effects in such detail is a major advantage of this phenotyping approach and may provide new insights into how different genes operate and cooperate during human facial development.

Moving forward: challenges and opportunities

One of the major challenges for GWAS of human craniofacial morphology – as with many other complex genetic traits – is identifying which of the associated variants are functional.

²⁷ Such identification is critical for elucidating the biological mechanisms through which genomic changes are translated into morphological outcomes during craniofacial development and growth. Unfortunately, because of the correlational structure of the genome, many variants at a given locus may show statistical association with a trait, but only a small number are likely to be functionally related to that trait.²⁸ These non-functional variants are associated by proxy. This problem is further exacerbated because virtually all variants from facial GWAS studies are located in the non-coding parts of the genome, whose function is still poorly understood. While many causative sequence changes likely affect gene expression via impacting function of cis-regulatory elements, predicting which of the trait-associated genetic variants will ultimately modulate cis-regulatory function is challenging. Some of these challenges can be addressed experimentally using high-throughput reporter or mutagenesis assays in cell culture models.²⁹ However, given the high cell-type selectivity of many regulatory elements and the fact that multiple cell types contribute to the craniofacial complex as development proceeds, the choice of cell culture models for such assays will be crucial to capture relevant putative regulatory variants. Even so, redundancies and complex combinatorial relationships within regulatory landscapes may complicate elucidation of the impact of individual variants on gene expression and ultimately connecting them to changes in facial shape. While the most direct test of function may involve installing select candidate functional variants in animal models such as the mouse, this approach is not only difficult to scale when many variants are involved, but in many cases may not even be valid. Indeed, the non-coding genomic space has been greatly remodeled during evolution since the split of primates from other mammals. Consequently, many human cis-regulatory regions, including those active in the human cranial neural crest cells, have not been deeply conserved during evolution and do not have orthologous sequences in the rodent genome.³⁰

There are other, more basic challenges that we face as a field. While we have made some major advances in phenotyping, the samples we have access to tend to be small, which limits our ability to detect variants with weak phenotypic effects or variants that are rare in the population. Discovery cohorts for facial GWAS have included between 1,800–7500 individuals. As a frame of reference, cohorts used in other common complex trait GWAS (e.g., height, BMI, psychiatric traits) often exceed over 100,000 individuals. Aside from the relatively small size of the existing facial GWAS cohorts, such cohorts are also relatively scarce. While there are numerous well-phenotyped datasets containing GWAS data, very few have any information pertaining to craniofacial morphology. For the existing datasets, in only a handful of instances are the raw underlying genotype and phenotype/image data able to be shared, making collaborations difficult. Moreover, inconsistent phenotyping among cohorts limits the ability to perform meta-analyses or independent replications. These factors limit our ability to put together large multicenter studies to achieve samples sizes with adequate power to fully investigate the genetic architecture of complex traits like facial shape. Nevertheless, there has been some progress. The FaceBase Consortium, for example, has made available individual-level genome-wide variants and 3D facial images on about 6000 individuals of European or African ancestry.³¹ As more sharable resources of this kind emerge, these limitations may soon be overcome.

As we learn more about the genomic underpinnings of human facial shape, the potential for applying this information to solve real world problems becomes a more concrete possibility. The ability, for example, to predict aspects of facial morphology or facial growth from genetic information could have numerous applications, in the clinic and beyond. The concept of personalized orthodontics, where the therapeutic approach is based in part on a patient's genetic profile, is already shifting from theory to reality.⁵ Genetic information may also eventually inform surgical planning to correct craniofacial malformations. While such possibilities are exciting to consider, it must be remembered that the prediction of complex genetic traits is highly problematic.³² This is because the path between genotype and phenotype is rarely ever straightforward. A person's facial morphology is not simply the sum product of their genes, but is also impacted by a wide-variety of non-genetic factors (e.g., prenatal effects, climate, diet, biomechanical forces). Moreover, there are likely to be extremely complex interactions at work. Thus, even if we had knowledge of how every variant in the genome affects the human face, we would still not have a complete picture. This is not a zero-sum effort, however, because predictive ability does not need to be at 100% to be of practical use. These are all things to keep in mind as we enter the era of precision medicine for the face.

Conclusions

Gene mapping studies in several different populations have now identified numerous genetic variants associated with normal-range human facial variation. Many of the genes implicated in these studies are active during craniofacial development and several have now been independently replicated. The associated variants tend to be in the non-coding parts of the genome, suggesting that they play a role in regulating transcription. While these results are exciting, our understanding of the genomic architecture of facial traits remains largely incomplete. There are both methodological and practical hurdles to advancement, including a lack of available datasets. Overcoming these challenges will require a coordinated and multidisciplinary effort.

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Fig. 1. Segmentation of the 3D facial surface into 63 modules (blue) and implicated genes/chromosomal locations according to broad facial regions from Claes et al. 2018. * Indicates genes that showed strong evidence of association during discovery but did not fully replicate.

Table 1:

Summary of GWAS studies focusing on quantitative human facial morphology

Study	Cohort	Study Design	Phenotypes	Key Outcomes
Patemoster et al. ¹⁵	UK - White 3D facial images	Discovery: N=2185 Replication: N=1622	3D and 2D linear distances (n=54); principal components of linear distances (n=14)	First published GWAS of facial morphology; Reported four genome-wide significant associations; Identified and replicated <i>PAX3</i> association with nasal root morphology
Liu et al. ¹⁶	European - White 3D and 2D facial images	Discovery: N=5388 Replication: N=5266	3D linear distances (n=36) ^a ; principal components (n=11); facial size (n=1)	Identified genome-wide significant associations implicating five gene, including <i>PAX3</i> , <i>TP63</i> and <i>PRDM16</i> ; Several orofacial cleft candidate SNPs associated with normal-range facial phenotypes.
Adhikari et al. ¹⁷	Admixed Latin American 3D and 2D facial images	Discovery: N=6275 Replication: N=501	3D linear distances and angles (n=8); graded categorical features (n=14)	First GWAS of facial morphology in a Latin American cohort; Identified several novel associations involving <i>DCHS2</i> , <i>SUPT3H/RUNX2</i> , <i>GLI3</i> , <i>PAX1</i> , and <i>EDAR</i> .
Shaffer et al. ¹⁸	US - White 3D facial images	Meta-Analysis N = 3118	3D linear distances (n=20)	Five genes/regions (including <i>MAFB</i> , <i>PAX9</i> , <i>ALX3</i> and <i>PAX1</i>) associated in 7 facial traits; Additional evidence of association from variants implicated in prior facial GWAS.
Cole et al. ¹⁹	East African 3D facial images	Discovery: N=3505 Replication: N=2390	3D linear distances (n=25); principal components of shape (n=6); facial size measures (n=3)	First GWAS of facial morphology in an African cohort; Identified novel associations (<i>SCHIP1</i> , <i>PDE8A</i>) with facial size; Associated genes showed expression in the developing mouse face.
Lee et al. ²⁰	US - White 3D facial images	Meta-Analysis N = 2187	Factors (n=23) derived from 276 3D linear distances	First GWAS to identify genome-wide significant associations with composite facial phenotypes; Implicated genes included <i>PARK2</i> and <i>FREM1</i>
Claes et al. ²¹	US - White 3D facial images	Discovery: N=2329 Replication: N=1719	Principal components of shape for 3D facial surface modules (n=63) derived from hierarchical segmentation	Introduced novel phenotyping approach designed to exploit the full shape information contained in 3D facial surface images; Identified 38 genome-wide significant loci, replicated 15 signals; Numerous developmentally important genes implicated (e.g. <i>TBX15</i> , <i>DLX6</i> , <i>SOX9</i>). Variants at the implicated loci showed evidence of involvement in the epigenetic regulation of cranial neural crest cells. In addition, variants implicated in prior GWAS showed evidence of association with similar phenotypic effects.
Crouch et al. ²²	UK - White 3D facial images	Discovery: N=1832 Replication: N=1567	Principal components of shape based on 3D facial surface morphology (n=40)	First GWAS to test extreme facial phenotypes to identify variants with larger effects; implicated genes included <i>MBTPS1</i> , <i>TNFRSF163</i> , and <i>PDCH1527</i>
Cha et al. ²³	Korean 2D facial images	Discovery: N=5643 Replication: N=1926	2D linear distances, angles and areas (n=85)	First published GWAS of facial shape in an Asian population; Identified five genome-wide significant associations, included two signals near genes (<i>HOXD1</i> - <i>MTX2</i> ; <i>SOX9</i>) reported in prior European GWAS.

^a replication involved measurements derived from 2D images