(1) Final Technical Report

I. Introduction

Human appearance is a remarkably variable set of physical traits that together comprise the summation of many different externally visible characteristics. Of all externally visible characteristics, facial appearance is paradoxically one of the most morphologically variable and individually distinctive features, and yet at the same time is the most recognizable visible characteristic of humans. Human facial features and facial expressions comprise the basis of much of social interaction, and the human brain has evolved the extraordinary capacity for virtually instantaneous individual recognition and friend-foe discrimination on the basis of facial features and facial expressions (Bruce & Young, 1986). Human facial appearance is largely genetically determined, though little is currently known about either the underlying genes or genetic architecture. Each of the many components that define overall facial shape and appearance are likely determined by a multiplicity of genes, with environmental variables exerting increasing influence over time. Nevertheless, the striking similarity of facial appearance within families, often across many generations, suggests that certain key genes may exert particularly large effects on overall appearance (reviewed in Project publication 1; Cole and Spritz, 2017).

A *ne plus ultra* goal of forensic science would be the ability to predict a virtual photographic representation of facial appearance from genomic DNA sequences derived from forensic specimens, so-called “Molecular Photofitting” (Frudakis, 2007). That may never be possible in full, due to major environmental and temporal influences on facial appearance. The purpose of this project was to conduct key basic research necessary to realize this goal, aiming to discover the genetic basis of facial appearance, which constitutes a fundamental body of knowledge needed to predict facial appearance from DNA sequences. Our studies leveraged an existing set of research subjects, samples, and facial shape data, as well major investment by the United States National Institutes of Health, enabling us to carry out a both large-scale studies of the heritability of facial shape characteristics (in an African-derived population; AFR) and to carry out a large-scale genome-wide association study GWAS) of the genetic underpinnings of facial shape (in a USA European-derived Caucasian population; EUR).

II. Review of Relevant Literature

Human facial morphology is defined by a remarkably complex assemblage of highly variable distinct anatomic structures that make each of us unique and distinguishable. Discovering both the genetic and environmental contributions and specific determinants of normal facial variation can help provide better understanding of both normal and abnormal human facial development.

Our current understanding of craniofacial biology revolves around genes and signaling pathways that drive embryonic development of the overall facial skeleton and connective tissues, primarily derived from cranial neural crest cells (NCC) and associated epithelia. These cranial NCC populations migrate, differentiate, and proliferate to form both cartilage and bone of the distinct facial prominences that later fuse to form the face. This complex and tightly regulated process is controlled by both intrinsic genetic cues and extrinsic signals provided by neighboring developing cell populations. Furthermore, the face grows and changes throughout postnatal life, including adulthood (Lande 1952, Formby et al. 1994), while facial skeleton and soft facial tissue growth are not always concordant (Subtelny 1959).

Aspects of facial morphology in general are highly heritable; even from a non-geneticist and non-quantitative point of view, it is obvious there is a higher degree of facial similarity between related individuals than unrelated individuals. The first genetic studies of human facial

morphology were heritability analyses that qualitatively interpreted facial data to support a genetic basis of human facial form (Rubbrecht 1939, Lebow and Sawin 1941, Hughes 1942, Kraus et al. 1959). Nevertheless, more quantitative approaches to measuring the heritability of human facial morphology have resulted in widely varying heritability estimates and conclusions, largely due to diverse study designs that make it difficult to directly compare results. These include differences in image capture methods (e.g. cephalograms, skulls, direct anthropometric measurements, 2D photographs, and 3D digital images), specific quantitative phenotypes used (i.e. distances, angles, proportions, multivariate measurements, and measures of size), different levels of subject relatedness (i.e. twins, families, and unrelated individuals), differences in statistical methods for estimating heritability (i.e. Falconer's formula, Holzinger's formula, parent-offspring regression, and linear mixed models with either pedigree-based or empirical genetic estimates of relatedness) and different types of estimates of heritability (i.e. heritability percentages, familial correlations, heritability explained by common genetic variation, and total narrow-sense heritability). In addition, differing non-genetic variables, such as age and ethnicity, can influence heritability estimates. Moreover, heritability is not static, as the specific genetic and environmental contributors and their impact may vary at different stages of life (Byard et al. 1983). Despite these difficulties, there is a high positive correlation in heritability estimates for the same facial traits among different studies, and the mean heritabilities of commonly studied facial traits are all over 50%, with no significant difference between cephalometric and anthropometric studies (Kohn 1991).

To date, five GWAS have been conducted in efforts to identify genes that influence normal facial shape variation in various populations, including Europeans (Paternoster et al. 2012) (Liu et al. 2012), Latin Americans (Adhikari et al. 2016), Bantu African children (Cole et al. 2016), and USA European-derived Caucasians (Shaffer et al. 2016). All five detected significant associations of common genetic variants with various facial morphometric traits. However, there has been remarkably little genetic overlap among the significant loci detected by these studies.

Nevertheless, despite limited success in identifying genetic determinants of facial shape, there has been great interest in prediction of facial appearance from DNA for forensic purposes. Current phenotype prediction models based of adult human height, a relatively simple (and the most well-studied) externally visible trait, are becoming more accurate with the inclusion of a large number of loci with proven genetic association (Aulchenko et al. 2009, Liu et al. 2014). In contrast, the current landscape of the genetics of normal facial shape variation is far sparser; thus, we are very far from being able to model the human face from DNA. Claims to currently be able to do so are spurious, being largely based on sex and genomic ancestry rather than actual genetic variables that determine facial shape, and thus are far from any reliable application (Claes et al. 2014a, Claes et al. 2014b, Fagertun et al. 2015). Genetic tests are already being marketed with claims as being able to predict the human face from forensic specimens. These tests have yet to be subjected to any rigorous external validation, and almost certainly have virtually nil positive predictive value. This constitutes a serious problem both for scientific understanding of facial genetic determinants and for forensic analysis, as the current situation may jeopardize credibility of the entire field.

III. Methods and Modifications to Original Research Design

All project deliverables were accomplished. Moreover, over the course of the project important opportunities presented that enabled major improvements over what was originally proposed, substantially benefitting the knowledge ultimately realized by the project. The originally proposed project deliverables were:

Deliverable 1: Formatting existing three-dimensional (3D) facial scans of 797 normal EUR subjects to .obj files.

Completed in project year 1. Of the 797 scans, 777 passed quality control (QC) checks. The 20 failed scans could not be used further.

Deliverable 2. Landmarking 3D facial scans of those normal EUR subjects.

Completed in project year 1. To accomplish this, we developed a revolutionary automated landmarking method for facial scans (Reported in Project publication 2; Li et al., 2017). This method applied 29 standard facial morphometric landmarks to the scans. The method is robust and can be implemented without high-performance computing resources. We validated the method using both direct comparison to manual landmarking on the same individuals and also analyses of the variation patterns and outlier patterns in a large independent set of automated and manual landmark data. This showed that automated landmark data are less variable, but more highly integrated and more reproducible than manual landmarks. Errors are readily detectable and can be easily fixed. The 777 scans that passed initial QC were subjected to automated landmarking; 682 passed detailed QC; the 95 failed scans were then subjected to manual landmarking.

Deliverable 3. Derivation of facial shape principal components (PCs) and standard measurements for the normal EUR subjects.

Completed in project year 2. The 777 landmarked scans were used to derive 25 standard inter-landmark 3D facial surface measurements and three global measures of facial size. Two subjects were measurement outliers and could not be used further. Five additional summary variables were derived from a principal components analysis (PCA) of whole face, together explaining ~70% of total facial variation. One additional summary variable was derived from a PCA of the most highly correlated mid-facial landmarks (explaining approximately 40% of total midface variation).

Deliverable 4. Genotyping of 384 SNPs (or comparable tagSNPs more appropriate to a EUR population) previously found to be genetically associated with facial shape parameters in a Tanzanian African (AFR) population

Completed in project year 2. This deliverable was expanded significantly, as we were presented with the opportunity for extraordinary leverage by incorporating our samples into a large GWAS of facial shape genetics in EUR subjects in collaboration with Profs. Seth Weinberg and Mary Marazita at the University of Pittsburgh, under their project sponsored by the National Institutes of Health FaceBase1 initiative (Reported in Project publication 3; Brinkley et al., 2016). Accordingly, we prepared genomic DNA samples from the 777 EUR subjects whose scans passed all QC and sent these to the Center for Inherited Disease Research (CIDR), operated at Johns Hopkins University, for secondary QC and genotyping of 964,193 SNPs on the Illumina OmniExpress+Exome v1.2 array plus 4,322 custom SNPs chosen in regions of interest based on previous studies of the genetic of facial variation. Data from our “Denver sample” were combined with those of 2447 unrelated EUR subjects (“Pittsburgh sample”) recruited as part of the FaceBase Consortium 3D Facial Norms Dataset (Weinberg et al., 2015). Initial genotype QC was carried out by CIDR and by the University of Washington Department of Biostatistics. Genotype data were further imputed to the 1000 Genomes reference panel (Phase 3), providing data for a total 34,985,077 variants genomewide (Reported in Project publication 4; Shaffer et al., 2016).

Deliverable 5. Genetic analysis to test association of these SNPs with corresponding facial shape parameters in the EUR population

Completed in project year 2 and NCE year 3. A total of 20 quantitative inter-landmark 3D surface measurements measured in common between the Denver and Pittsburgh subject cohorts (Fig. 1) were subjected to genomewide genetic association analysis independently, and results from the two subject cohorts, totaling 3118 normal EUR subjects, were combined by meta-analyses.

Fig. 1. Facial shape measures used in EUR GWAS. From Shaffer et al., 2016 (Project publication 4)

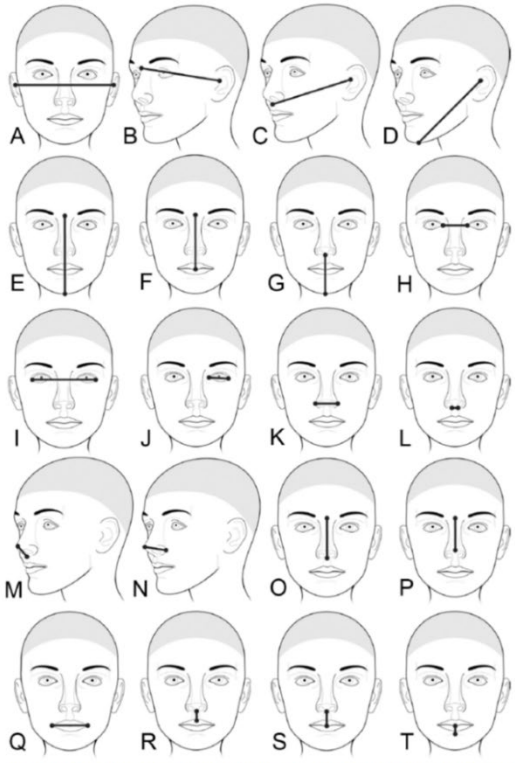

Fig 1. Set of 20 linear distance measurements used in the current study. (A) Cranial base width, (B) Upper facial depth*, (C) Middle facial depth*, (D) Lower facial depth*, (E) Morphological facial height, (F) Upper facial height, (G) Lower facial height, (H) intercanthal width, (I) Outercanthal width, (J) Palpebral fissure length*, (K) Nasal width, (L) Subnasal width, (M) Nasal Protrusion, (N) Nasal ala length*, (O) Nasal height, (P) Nasal Bridge Length, (Q) Labial fissure length, (R) Philtrum length, (S) Upper lip height, and (T) Lower lip height. Measurements with an asterisk (*) are bilateral, but only the left side is shown in the figure.

We observed genome-wide significant associations ($p < 5 \times 10^{-8}$) for cranial base width at 14q21.1 and 20q12, intercanthal width at 1p13.3 and Xq13.2, nasal width at 20p11.22, nasal ala length at 14q11.2, and upper facial depth at 11q22.1. Several genes in the associated regions are known to play roles in craniofacial development or in syndromes affecting the face: MAFB, PAX9, MIPOL1, ALX3, HDAC8, and PAX1. We also tested genotype-phenotype associations reported in two previous genomewide studies and found limited evidence of replication for nasal ala length and SNPs in CACNA2D3 and PRDM16. These results indicate that common variants in regions harboring genes of known craniofacial function contribute to normal variation in human facial features. Improved understanding of the genes associated with facial morphology in healthy individuals can provide insights into the pathways and mechanisms controlling normal and abnormal facial morphogenesis (Project publication 4; Shaffer et al., 2016).

Deliverable 6. Deposition of all project data in appropriate scientific data repositories, within the framework of those repositories' operational guidelines

Completed in project year 2. All project genotype data were deposited in the Database of Genotypes and Phenotypes (dbGaP; https://www.ncbi.nlm.nih.gov/gap) [accession phs000949.v1.p1]. All project derived phenotype measures and 3D facial scans were deposited in the NIDCR FaceBase Hub (https://www.facebase.org/).

IV. Findings

The expansion of the proposed project from testing selected candidate genes to a full-scale GWAS enabled a greatly expanded range of analyses, including genomewide screening of candidate genes, genomewide discovery of previously unknown genes, comparison across GWAS datasets, investigation of heritability of facial shape, and the effect of environmental influences on facial shape. Each yielded important new data, discussed here.

A. Genomewide screening of candidate genes.

In the EUR facial shape GWAS, we first attempted to replicate the main findings from the two previous of facial shape in EUR (see Project publication 4; Shaffer et al., 2016). As shown in Table 1, we tested previously implicated SNPs against traits from our dataset that capture similar aspects of morphology as previously reported genotype-phenotype associations, though directly analogous comparisons were not possible in all cases.

Table 1. Testing of previously reported genomewide significant SNPs in EUR (from Shaffer et al., 2016)

	Published GWAS Results						Current Results		
Study	**Locus**	**Candidate gene**	**SNP**	**Minor allele (MAF)**	**Beta (p)**	**Associated phenotype(s)**	**Closest phenotype (s) in our dataset**	**Minor allele (MAF)**	**Beta (p)**
Paternoster et al. [17]	12q21.3	*TMTC2*	rs10862567	T (0.31)	0.181 ($4.4x10^{-8}$)	Position of the right endocanthion point	N/A	T (0.32)	N/A
	2q36	*PAX3*	rs7559271	G (0.38)	0.169 ($2.2x10^{-10}$)	Nasion to mid-endocanthion distance	Intercanthal width (Fig 1H)	G (0.37)	0.066 (0.392)
	3p14.3	*CACNA2D3*	rs1982862	A (0.12)	-0.257 ($1.8x10^{-8}$)	Pronasale to alare distance	Nasal ala length (Fig 1N)	A (0.16)	-0.297 (0.018)
	5q12	*C5orf64*	rs11738462	A (0.17)	-0.204 ($1.8x10^{-8}$)	Pronasale to alare distance	Nasal ala length (Fig 1N)	A (0.18)	0.028 (0.818)
Liu et al. [18]	1p36.3	*PRDM16*	rs4648379	T (0.28)	-0.260 ($1.1x10^{-8}$)	Pronasale to alare distance	Nasal ala length (Fig 1N)	T (0.28)	-0.447 ($1.7x10^{-5}$)
	2q36	*PAX3*	rs974448	G (0.17)	0.290 ($1.6x10^{-8}$)	Nasion to orbit distance [a]	Intercanthal width (Fig 1H)	G (0.17)	0.171 (0.002)
	3q28	*TP63*	rs17447439	G (0.04)	-0.910 ($4.4x10^{-8}$)	Distance between the orbits [a]	Intercanthal width (Fig 1H)	G (0.04)	0.043 (0.758)
							Outercanthal width (Fig 1I)	G (0.04)	0.261 (0.273)
	5q35.1	*C5orf50*	rs6555969	T (0.33)	0.410 ($1.2x10^{-9}$)	Nasion to zygion distance	Upper facial depth (Fig 1B)	T (0.33)	0.615 (0.005)
					0.260 ($2.3x10^{-9}$)	Nasion to orbit distance [a]	Intercanthal width (Fig 1H)	T (0.33)	0.159 (0.049)
	10q25.1	*COL17A1*	rs805722	T (0.19)	0.290 ($4.0x10^{-8}$)	Nasion to orbit distance [a]	Intercanthal width (Fig 1H)	T (0.18)	0.047 (0.628)

[a] orbit landmark measured from MRI at the approximate location of the pupil

For Paternoster et al. [17], we attempted to test three of their four reported genomewide significant associations, two of which involved nasal ala length. In our data, nasal ala length

showed nominally significant association (p = 0.018) with rs1982862, an intronic variant in CACNA2D3. Conversely, we found no evidence of association between this measure and rs11738462, an intronic variant in C5orf64. The previously observed association between PAX3 and the position of nasion relative to the orbits could not be tested directly. However, we found no evidence of association between the implicated SNP rs7559271 and intercanthal width, which captures aspects of interorbital septum morphology. As a further exploratory analysis we also tested association between rs7559271 and several vertical or projective measurements involving nasion, finding no significant associations were found for any of these traits. For Liu et al. [18], we attempted to test each of their six previously reported genomewide significant associations (Table 2). We observed suggestive association (p = 1.70 x 10−5) between nasal ala length and rs4648379, an intronic variant in PRDM16. We also observed nominally significant association between rs6555969, a SNP near C5orf50, and upper facial depth (p = 0.005), which is a reasonable approximation of the zygion-nasion distance reported by Liu et al. [18]. To test the association between interorbital distance and rs17447439, an intronic variant in TP63, we used measures of intercanthal and outercanthal width; however, we did not observe any association with either measure. Finally, Liu et al. [18] reported associations between SNPs in PAX3, C5orf50, and COL17A1 and the position of nasion relative to the orbits. We tested these three SNPs in our dataset against intercanthal width, a trait involving roughly similar anatomical components. We found nominally significant associations between rs974448 (PAX3, p = 0.002) and rs6555969 (C5orf50, p = 0.049) and intercanthal width. While these observations provide some evidence of replication of previously reported associations; however, the strengths of the observed associations are quite weak. For some other previously reported associations, we observed no evidence of replication at all.

B. Genomewide discovery of previously unknown genes

The expansion of the project to a full GWAS of facial shape in EUR provided important opportunities for discovery of entirely unknown genes that affect facial shape. As shown in Table 2, in the EUR facial shape GWAS (Project publication 4; Shaffer et al., 2016), we observed seven associations in five traits that exceeded the threshold for genomewide significance (p < 5 x 10−8). One of the associations also exceeded our studywide significance threshold of p < 5 x 10−9, calculated based on 10 independent traits. Due to the large number of traits, we limit presentation of results here to genome-wide significant signals.

Table 2. Genomewide significant meta-analysis results for five traits in EUR (from Shaffer et al., 2016)

				Pittsburgh Sample				Denver Sample				Meta-Analysis
Trait	**SNP**	**Locus**	**Minor allele**	**MAF**	**N**	**Beta (se)[a]**	**p**	**MAF**	**N**	**Beta (se)[a]**	**p**	**p**
Cranial base width (Fig 1A)	rs17106852	14q21.1 (38038468)	G	0.106	2368	-1.104 (0.205)	7.91×10^{-8}	0.097	671	-0.858 (0.406)	3.51×10^{-2}	**1.01×10^{-8}**
	rs6129564	20q12 (38904203)	A	0.116	2368	-1.210 (0.193)	**4.07×10^{-10}**	0.100	671	-0.449 (0.412)	2.77×10^{-1}	**1.65×10^{-9}**
Intercanthal width (Fig 1H)	rs619686	1p13.3 (110218761)	G	0.056	2426	-0.763 (0.163)	3.12×10^{-6}	0.052	671	-0.536 (0.186)	4.12×10^{-3}	**4.70×10^{-8}**
	rs11093404	Xq13.2 (72289467)	A	0.243	2426	0.427 (0.075)	**1.31×10^{-8}**	0.248	671	0.073 (0.075)	3.32×10^{-1}	**4.16×10^{-8}**
Nasal width (Fig 1K)	rs2424399	20p11.22 (21632545)	C	0.235	2429	0.377 (0.070)	9.53×10^{-8}	0.300	671	0.177 (0.098)	7.04×10^{-2}	**2.62×10^{-8}**
Nasal ala length (Fig 1N)	rs8007643	14q11.2 (21365801)	T	0.067	2426	1.064 (0.186)	**1.19×10^{-8}**	0.069	671	0.221 (0.216)	3.07×10^{-1}	**3.36×10^{-8}**
Upper facial depth (Fig 1B)	rs12786942	11q22.1 (101394765)	T	0.119	2368	1.429 (0.317)	6.95×10^{-6}	0.105	670	1.674 (0.523)	1.43×10^{-3}	**4.59×10^{-8}**

[a] Beta values determined based on minor allele

We observed two significant associations for cranial base width: one at 14q21.1 (top SNP rs79272428, p = 1.01 x 10−8) and the other at 20q12 (top SNP rs6129564, p = 1.65 x 10−9). Notably, the chromosome 20 association exceeded our strict threshold for studywide statistical significance. For intercanthal width, we observed two significant associations: one at 1p13.3 (top SNP rs619686, p = 4.70 x 10−8,) and the other at Xq13.2 (top SNP rs11093404, 4.16 x 10−8). There were also significant associations with nasal width at 20p11.22 (rs2424399, p = 2.62 x 10−8) and nasal ala length at 14q11.2 (top SNP rs8007643, p = 3.36 x 10−8). We observed a second independent peak on chromosome 20 for nasal width located 371kb upstream of the main peak. The second peak remained (top SNP rs80186620, p = 5.32x10-6) after conditional association analysis adjusting for the effects of rs2424399 on nasal width. Finally we observed a significant association with upper facial depth at 11q22.1 (top SNP rs12786942, p = 4.59 x 10−8). For all of the above associations, the results were driven primarily by the larger Pittsburgh dataset. The cranial base width (14q21.1), intercanthal width (1p13.3) and upper facial depth associations were at least nominally significant (p < 0.05) in both the Pittsburgh and Denver datasets.

C. Comparison across GWAS datasets

In parallel to the GWAS of facial shape in EUR, we carried out an analogous GWAS of facial shape in a Bantu AFR population. These data were analyzed in parallel and compared with the EUR data (Project publication 5; Cole et al., 2016). As shown in Table 3, the AFR study (which additionally included an independent Bantu AFR replication cohort) identified two novel genomewide significant associations, both of which replicated in the AFR replication cohort, surpassing a strict genomewide significance criterion for an African population (P < 2.50 x 10−8) plus independent replication (P < 0.05), with no significant inter-study heterogeneity (I2 < 50%), and surpassed a stringent genomewide meta-analysis significance threshold corrected for 9 effectively independent phenotypes tested (P < 2.50 x 10−8 / 9 = 2.78 x 10−9).

Table 3. GWAS, replication, and meta-analysis results of replicated AFR associations (from Cole et al., 2016)

SNP	Trait	CHR	NT	Gene	Effect Allele	GWAS MAF	GWAS P-value	GWAS β	GWAS SE[c]	Replication MAF[a]	Replication 1-sided P-value[a,b]	Meta-analysis I² heterogeneity	Meta-analysis P-value[b]	Meta-analysis β	Meta-analysis SE[c]
rs79909949	Centroid Size	3	159774689	*SCHIP1*	C	0.021	9.56E-09	-4.32	0.75	0.0044	1.80E-03	0.0	6.58E-11	-4.47	0.70
rs12909111	Allometry	15	85061095	*PDE8A*	G	0.30	2.36E-07	0.0027	0.00052	0.31	1.53E-03	12.0	2.52E-09	0.0023	0.00040
rs12908400	Allometry	15	85029945	*PDE8A*	G	0.30	1.92E-07	0.0027	0.00051	0.30	1.03E-02	35.6	2.36E-06	0.0021	0.00040
rs7836044	STO_SL	8	136880907	Intergenic	T	0.47	5.69E-06	-0.17	0.032	0.50	3.08E-02	46.1	2.70E-06	0.14	0.025
rs139879053	PC1	1	23313306	*HNRNPR*	T	0.024	3.63E-07	-0.0084	0.0016	0.012	2.04E-02	8.0	2.73E-06	-0.0078	0.0014
rs148037459	EX_R_EX_L	9	93212785	*WNK2*	A	0.086	4.32E-06	-0.42	0.091	0.092	3.58E-03	0.0	5.88E-06	-0.39	0.073
rs2817419	EX_R_EX_L	6	50845193	*TFAP2B*	G	0.37	4.75E-07	-0.27	0.053	0.36	2.18E-02	0.5	7.28E-06	-0.23	0.043
rs35965172	EN_EX	6	50849828	2 kb downstream *TFAP2B*	T	0.31	3.57E-06	-0.11	0.025	0.32	2.39E-02	0.0	4.82E-07	-0.097	0.020
rs114189713	CH_R_CH_L	7	154832074	*DPP6*	A	0.039	1.84E-07	-0.62	0.12	0.037	2.64E-02	45.7	8.35E-06	-0.47	0.090
rs7627283	PC2	3	16192201	*GALNT15*	A	0.39	1.02E-06	0.0023	0.00048	0.37	2.91E-02	45.0	4.01E-07	0.0018	0.00040
rs75004472	LI_SL	15	27252846	*GABRG3*	G	0.14	3.25E-06	0.18	0.041	0.15	2.40E-02	32.0	7.47E-07	0.15	0.031
rs12112855	PC3	7	155796005	*RBM33*	T	0.076	1.55E-06	-0.0040	0.00082	0.071	4.08E-02	37.5	8.08E-07	-0.0030	0.00060
rs73936436	SBAL_R_SBAL_L	2	65216165	10kb upstream *ACTR2*	T	0.036	6.02E-06	-0.43	0.094	0.039	1.50E-02	24.5	9.74E-07	-0.32	0.067
rs61448486	N_MEN	2	72243866	*EXOC6B*	A	0.36	3.82E-06	-0.14	0.031	0.37	3.66E-02	13.5	9.92E-07	-0.12	0.025

[a]Replication MAF and P-value are combined from the meta-analysis of the two individual replication studies by cameta
[b]Replication and meta-analysis P-values are 1-sided
[c]SE, standard error of *β*

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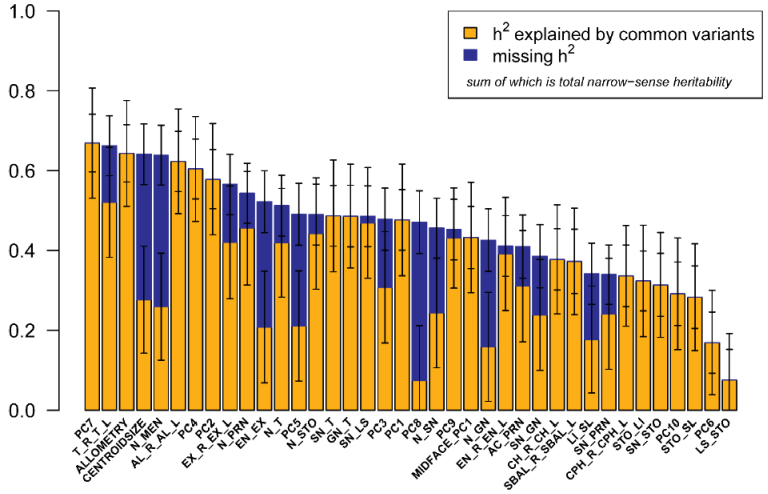
The first of the novel associations in AFR was of SNPs in the SCHIP1 region of chromosome 3q25.33 (chr3:159,774,689–159,960,389) with centroid size, a measure of overall facial size that is uncorrelated with variables of shape. The second association was of SNPs in the PDE8A region of chromosome 15q25.3 (chr15:84,923,649–85,161,983; Fig 5A and S6 Fig) with the allometry variable, which represents a complex scaling relationship between size and shape. Moreover, 49 of the allometry-associated SNPs in the PDE8A region also showed marginal association with various facial measurement phenotypes. 11 additional loci showed suggestive association (P < 10-5) with various other facial measurement phenotypes.

Importantly, comparison of the AFR and EUR GWAS results showed absolutely no cross-replication. That is, no association signals that were at least suggestively significant in one population yielded any apparent association signal in the other. This suggests that either the specific genetic loci that affect facial shape in AFR are different than those in EUR, and vice-versa, or that a very large number of genes affect facial shape, and that both studies were underpowered and yielded only a small subset of the total loci.

D. Heritability of facial shape

The availability of a large dataset of individuals' facial measurements derived from 3D scans and corresponding whole-genome genotype data enabled, for the first time, detailed assessment of the narrow-sense heritability (h^2) underlying these measurements using a linear mixed model variance components approach; essentially, the proportion of these phenotypes that is attributable to genes. These analyses were carried out using the Bantu AFR dataset from 3636 individuals (Project publication 6; Cole et al., 2017). As shown in Fig. 2, our significant estimates for narrow sense heritability of specific facial traits range from 28% - 67%, with horizontal measures being slightly more heritable than vertical or depth measures. Furthermore, for over half of facial traits >90% of narrow-sense heritability can be explained by common genetic variation. We also found high absolute genetic correlation between most traits, indicating large overlap in underlying genetic loci. Not surprisingly, traits measured in the same physical orientation (i.e., both horizontal or both vertical) have high positive genetic correlations, whereas traits in opposite orientations have high negative correlations. The complex genetic architecture of facial shape informs our understanding of the intricate relationships among different facial features as well as overall facial development.

Fig. 2. Heritability of 38 human facial traits. Barplot represents h^2_g (yellow), missing h^2 (blue), and total h^2 (yellow and blue) for all traits analyzed (see Fig. 1). From Project publication 6; Cole et al., 2017.



E. Effect of environmental influences on facial shape

The availability of large facial shape and corresponding genomewide datasets, together with data on stature and epidemiologic information from the same subjects, enabled us to analyze the environmental effects of socioeconomic status, a proxy for nutrition, on facial shape in the AFR cohort. Variation in the shape of the human face and in stature is determined by complex interactions between genetic and environmental influences. One such environmental influence is malnutrition, a well-known determinant of short stature due to growth faltering. In our large Bantu AFR sample of children aged 5-19, we correlated high-resolution genomic data, anthropometric measures, and 3D facial images. By Genome-Wide Complex Trait Analysis, we partitioned genetic and environmental variance for growth outcomes and facial shape. Children with short stature (growth faltering) have faces that look like those of older and taller children and in a direction opposite to the expected allometric trajectory and in ways predicted by the environmental portion of covariance at the community and individual levels. The environmental variance for facial shape varies subtly but significantly among communities while genetic differences were minimal. These results reveal that facial shape preserves information about exposure to undernourishment with important implications for refining assessments of nutritional status in children and the developmental-genetics of craniofacial variation alike (reported in Project publication 7; Cole et al., 2018).

Conclusions

The human face comprises an assemblage of multifactorial complex traits, with clear genetic components and known environmental factors playing key roles in its development and maturation throughout life. Recent advances in methodology for deriving accurate and precise facial traits have allowed for several large genomic studies that have provided new insights into the heritability and genes involved in normal facial variation.

Facial trait characteristics can be represented by measurements between anatomic landmarks, as well as more complex facial shape phenotypes represented as principal components. It is clear that the genetic components underlying such facial trait phenotypes are high, with h^2 estimates of 28% - 67%. Furthermore, for over half of facial traits >90% of narrow-sense heritability can be explained by common genetic variation, with high absolute genetic correlation between most traits, indicating large overlap in underlying genetic loci.

Nevertheless, the results of GWAS to discover genes involved in determining human facial shape have been striking in their lack of concordance across studies. To date, five GWAS of facial shape measures have been conducted in various populations, including Europeans (Paternoster et al. 2012) (Liu et al. 2012), Latin Americans (Adhikari et al. 2016), Bantu African children (Cole et al. 2016), and USA European-derived Caucasians (Shaffer et al. 2016). All five detected genomewide significant associations of common genetic variants with various facial morphometric traits (Table 1). However, there has been almost no cross-replication of the significant loci detected by these studies. Only association of PAX3 with different measures of nasion position have been replicated at genome-wide significance in more than one facial shape GWAS, though there has been some nominal replication of previous findings at the $P < 0.05$ level. The rs7559271 SNP identified in the first facial shape GWAS in Europeans (Paternoster et al. 2012) was replicated in a second facial shape GWAS in Europeans (Liu et al. 2012), though not in the third, of USA European-derived Caucasians (Schaffer et al. 2016). The GWAS of facial shape in Latin Americans aimed to replicate loci previously implicated in non-syndromic cleft lip/cleft palate (NSCLP), as close relatives of NSCLP patients may exhibit atypical facial shape (Weinberg et al. 2009, Boehringer et al. 2011). These investigators confirmed association of normal facial shape phenotypes with four NSCLP loci (Adhikari et al. 2016), including rs2235371 in IRF6 with brow ridge protrusion, rs13041247 near MAFB, with nasal root breadth,

rs1873147 near TPM1 with nasal root breadth, and rs560426 in ABCA with nose wing breadth. The GWAS of USA European-derived Caucasians replicated 5 SNPs at nominal significance ($P < 0.05$) identified in previous GWAS for comparable facial traits (Shaffer et al. 2016). These included rs1982862 in CACNA2D3, rs4648379 in PRDM16, rs6555969 near C5orf50, rs974448 near PAX3, and rs6555969 near C5orf50.

There are many possible explanations for such limited overlap of genetic associations found among these different facial shape GWAS. The most obvious is that biology of facial shape differences may be different in different populations, with the relevant causal variants driving association of shape differences in one population being absent (or perhaps irrelevant) in other populations. Alternatively, the causal variants may be present in multiple populations, but there may be differing magnitudes of effect or minor allele frequency in different cohorts that prevent those variants from reaching statistical significance in every study. Similarly, it may be that all studies to date have inadequate statistical power to sample a large fraction of the causation of facial shape variation, and so each study simply pulls out only a small fraction of the total relevant genetic variation. A large number of technical differences pertaining to imaging, landmarking, phenotyping, and adjustment for confounding factors among studies could also contribute to inter-study variability and result in non-overlapping results.

Despite the paucity of compelling evidence supporting specific genetic loci as major determinants of specific facial shape phenotypes in humans, there has been strong perception of need in the forensic community, perhaps underlain by faith in other DNA forensic tests. That situation has led to development and premature marketing of DNA-based tests that claim to predict facial appearance based on DNA. In fact, the principal basis for such tests are markers that are correlated with sex and ancestry, with little or no ability to predict facial appearance at the individual level. Moreover, there is abundant evidence that human facial appearance is strongly influenced by environmental factors and of course by chronologic age, and that genetics represents only one of the factors governing facial shape and appearance. As yet, there has been no rigorous external validation of claimed genetic-based tests of facial prediction, which likely have very low positive predictive value. There is real danger that unrealistic claims based on currently available tests may undermine faith in the entire field and stifle basic research that might serve to improve on current capabilities.

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(2) Summary

Human appearance is a remarkably variable set of physical traits that together comprise the summation of many different externally visible characteristics. Of all externally visible characteristics, facial appearance is paradoxically one of the most morphologically variable and individually distinctive features, and yet at the same time is the most recognizable visible characteristic of humans. Human facial appearance is largely genetically determined, though little is currently known about either the underlying genes or genetic architecture. Each of the many components that define overall facial shape and appearance are likely determined by a multiplicity of genes, with environmental variables exerting increasing influence over time.

A ne plus ultra goal of forensic science would be the ability to predict a virtual photographic representation of facial appearance from genomic DNA sequences derived from forensic specimens. That may never be possible in full, due to major environmental and temporal influences on facial appearance. Nevertheless, basic work necessary to enable genetic predictive goals is the discovery of genes that underlie facial appearance. Our studies were carried out in collaboration with the FaceBase1 consortium of the National Institutes of Health, and included large-scale studies of the heritability of facial shape characteristics (in an African-derived population; AFR) and a large-scale genome-wide association study (GWAS) of the genetic underpinnings of facial shape (in a USA European-derived Caucasian population; EUR).

To date, five GWAS have been conducted in efforts to identify genes that influence normal facial shape variation in various populations, including Europeans (Paternoster et al. 2012) (Liu et al. 2012), Latin Americans (Adhikari et al. 2016), Bantu African children (Cole et al. 2016), and USA European-derived Caucasians (Shaffer et al. 2016). All five detected significant associations of common genetic variants with various facial morphometric traits. However, there has been remarkably little genetic overlap among the significant loci detected by these studies. Nevertheless, despite limited success in identifying genetic determinants of facial shape, there has been great interest in prediction of facial appearance from DNA for forensic purposes. Unfortunately, claims to currently be able to predict facial appearance from forensic DNA specimens are spurious, being largely based on sex and genomic ancestry rather than actual genetic variables that determine facial shape, and thus are far from any reliable application (Claes et al. 2014a, Claes et al. 2014b, Fagertun et al. 2015). Genetic tests marketed with claims as being able to predict the human face from forensic specimens are thus premature, having no rigorous external validation, and likely very low positive predictive value. This constitutes a serious problem both for scientific understanding of facial genetic determinants and for forensic analysis, as the current situation may jeopardize credibility of the entire field.

Results and Findings

Facial 3D scans and DNA samples from 797 unrelated EUR subjects (the ‘Denver’ cohort) were processed; a total 777 passed all quality control procedures. Scans were subjected to automated landmarking using a novel procedure (Li et al., 2017), which applied 29 standard facial morphometric landmarks to the scans. 682 passed detailed QC; the 95 failed scans were then subjected to manual landmarking. The 777 landmarked scans were used to derive 25 standard inter-landmark 3D facial surface measurements and three global measures of facial size; two subjects were measurement outliers and could not be used further. Five additional summary variables were derived from a principal components analysis (PCA) of whole face, together explaining ~70% of total facial variation. One additional summary variable was derived from a PCA of the most highly correlated mid-facial landmarks (explaining approximately 40% of total midface variation).

Data and DNA from the 775 Denver samples were added to those of 2447 additional unrelated EUR subjects (the 'Pittsburgh' cohort) for whom 3D scans, measurements, and DNA were available under the NIH FaceBase 1 initiative (Brinkley et al., 2016). DNA samples were submitted the Center for Inherited Disease Research (CIDR), operated at Johns Hopkins University, for secondary QC and genotyping of 964,193 SNPs on the Illumina OmniExpress+Exome v1.2 array plus 4,322 custom SNPs chosen in regions of interest based on previous studies of the genetic of facial variation. Initial genotype QC was carried out by CIDR and by the University of Washington Department of Biostatistics. Genotype data were further imputed to the 1000 Genomes reference panel (Phase 3), providing data for a total 34,985,077 variants genomewide.

A total of 20 standard quantitative inter-landmark 3D surface measurements measured in common between the Denver and Pittsburgh subject cohorts were subjected to genomewide genetic association analysis independently, and results from the two subject cohorts, totaling 3118 normal EUR subjects, were combined by meta-analyses. We observed genome-wide significant associations ($p < 5 \times 10{-}8$) for cranial base width at 14q21.1 and 20q12, intercanthal width at 1p13.3 and Xq13.2, nasal width at 20p11.22, nasal ala length at 14q11.2, and upper facial depth at 11q22.1. Several genes in the associated regions are known to play roles in craniofacial development or in syndromes affecting the face: MAFB, PAX9, MIPOL1, ALX3, HDAC8, and PAX1. We also tested genotype-phenotype associations reported in two previous genomewide studies and found limited evidence of replication for nasal ala length and SNPs in CACNA2D3 and PRDM16. These results indicate that common variants in regions harboring genes of known craniofacial function contribute to normal variation in human facial features. Improved understanding of the genes associated with facial morphology in healthy individuals can provide insights into the pathways and mechanisms controlling normal and abnormal facial morphogenesis (Shaffer et al., 2016).

In parallel to the GWAS of facial shape in EUR, we carried out an analogous GWAS of facial shape in a Bantu AFR population. These data were analyzed in parallel and compared with the EUR data from the present project (Cole et al., 2016). The AFR study (which additionally included an independent Bantu AFR replication cohort) identified two novel genomewide significant associations, in the SCHIP1 region of chromosome 3q25.33 (chr3:159,774,689–159,960,389) with centroid size (a measure of overall facial size that is uncorrelated with variables of shape) and in the PDE8A region of chromosome 15q25.3 (chr15:84,923,649–85,161,983; Fig 5A and S6 Fig) with the allometry variable (which represents a complex scaling relationship between size and shape). Moreover, 49 of the allometry-associated SNPs in the PDE8A region also showed marginal association with various facial measurement phenotypes. 11 additional loci showed suggestive association ($P < 10\text{-}5$) with various other facial measurement phenotypes. Nevertheless, comparison of the AFR and EUR GWAS results showed absolutely no evidence of cross-replication.

The availability of a large dataset of individuals' facial measurements derived from 3D scans and corresponding whole-genome genotype data enabled, for the first time, detailed assessment of the narrow-sense heritability (h^2) underlying these measurements. These analyses were carried out using the Bantu AFR dataset from 3636 individuals (Cole et al., 2017), yielding significant estimates for narrow sense heritability of specific facial traits range from 28% - 67%, with horizontal measures being slightly more heritable than vertical or depth measures. Furthermore, for over half of facial traits >90% of narrow-sense heritability can be explained by common genetic variation. There was high absolute genetic correlation between most traits, indicating large overlap in underlying genetic loci. The complex genetic architecture of facial shape informs our understanding of the intricate relationships among different facial features as well as overall facial development.

Finally, the availability of large facial shape and corresponding genomewide datasets, together with data on stature and epidemiologic information from the same subjects, enabled us

to analyze the environmental effects of socioeconomic status, a proxy for nutrition, on facial shape in the AFR cohort. We correlated high-resolution genomic data, anthropometric measures, and 3D facial images. By Genome-Wide Complex Trait Analysis, we partitioned genetic and environmental variance for growth outcomes and facial shape. Children with short stature (growth faltering) have faces that look like those of older and taller children and in a direction opposite to the expected allometric trajectory and in ways predicted by the environmental portion of covariance at the community and individual levels. The environmental variance for facial shape varies subtly but significantly among communities while genetic differences were minimal. These results reveal that facial shape preserves information about exposure to undernourishment with important implications for refining assessments of nutritional status in children and the developmental-genetics of craniofacial variation alike (Cole et al., 2018).

Conclusions

The human face comprises an assemblage of multifactorial complex traits, with clear genetic components and known environmental factors playing key roles in its development and maturation throughout life. Recent advances in methodology for deriving accurate and precise facial traits have allowed for several large genomic studies that have provided new insights into the heritability and genes involved in normal facial variation. Facial trait characteristics can be represented by measurements between anatomic landmarks, as well as more complex facial shape phenotypes represented as principal components. It is clear that the genetic components underlying such facial trait phenotypes are high, with h2 estimates of 28% - 67%. Furthermore, for over half of facial traits >90% of narrow-sense heritability can be explained by common genetic variation, with high absolute genetic correlation between most traits, indicating large overlap in underlying genetic loci.

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Despite the paucity of compelling evidence supporting specific genetic loci as major determinants of specific facial shape phenotypes in humans, there has been strong perception of need in the forensic community, perhaps underlain by faith in other DNA forensic tests. That situation has led to development and premature marketing of DNA-based tests that claim to predict facial appearance based on DNA. In fact, the principal basis for such tests are markers that are correlated with sex and ancestry, with little or no ability to predict facial appearance at the individual level; hardly any markers have thus far reliably been associated with facial shape, and those that have exert relatively modest effects. Accordingly, while there has as-yet been no rigorous external validation of claimed genetic-based tests of facial prediction, current tests seem likely to have very low positive predictive value. Moreover, genetics are only one of the factors that govern human facial appearance: there is abundant evidence that human facial appearance is strongly influenced by environmental factors and of course by chronologic age. It thus may never be feasible to truly reliably predict human facial appearance from DNA. Whether that is true or not, there is real danger that unrealistic claims based on currently available tests may undermine faith in the entire field and stifle basic research that might serve to improve on current capabilities.

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(3) Abstract.

The human face comprises an assemblage of multiple multifactorial complex traits, each with clear genetic components as well as known environmental factors play key roles in the lifelong development and maturation of facial shape and appearance. Recent advances in methodology for deriving accurate and precise facial traits have allowed for large-scale genomic studies that have provided new insights into the heritability and specific genes involved in normal facial variation. The proposed project entailed genotyping candidate single-nucleotide polymorphisms (SNPs) that might be involved in facial shape in 797 European-derived white (EUR) subjects for whom we had existing three-dimensional facial scans and DNA samples. In fact, we were able to include these subjects in a collaborative large-scale genomewide association study (GWAS) of human facial shape in the EUR population. We conducted genome-wide association meta-analyses of 20 quantitative facial measurements derived from t3D surface images of a total 3118 healthy individuals of EUR ancestry. Subjects were genotyped for almost one million genotyped SNPs and were imputed to the 1000 Genomes reference panel (Phase 3), for almost 35,000,000 total SNPs. Detailed analyses showed heritability of facial trait measurements ranging from 28% - 67%, with horizontal measures being slightly more heritable than vertical or depth measures. Furthermore, for over half of facial traits >90% of narrow-sense heritability can be explained by common genetic variation. We also found high absolute genetic correlation between most traits, indicating large overlap in underlying genetic loci. We observed genome-wide significant associations for cranial base width at 14q21.1 and 20q12, intercanthal width at 1p13.3 and Xq13.2, nasal width at 20p11.22, nasal ala length at 14q11.2, and upper facial depth at 11q22.1. Several genes in the associated regions are known to play roles in craniofacial development or in syndromes affecting the face: MAFB, PAX9, MIPOL1, ALX3, HDAC8, and PAX1. We also tested genotype-phenotype associations reported in two previous genomewide studies and found evidence of replication for nasal ala length and SNPs in CACNA2D3 and PRDM16. In general, there has been limited cross-replication of loci associated with facial shape parameters across different GWAS. Furthermore, environmental influences, sex, and age play major roles in determining facial shape and appearance. This raises significant concerns about the validity and utility of current tests that claim to predict facial appearance from forensic DNA specimens.