Facial masculinity does not appear to be a condition-dependent male ornament and does not reflect MHC heterozygosity in humans

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Recent studies have called into question the idea that facial masculinity is a condition-dependent male ornament that indicates immunocompetence in humans. We add to this growing body of research by calculating an objective measure of facial masculinity/femininity using 3D images in a large sample (n = 1,233) of people of European ancestry. We show that facial masculinity is positively correlated with adult height in both males and females. However, facial masculinity scales with growth similarly in males and females, suggesting that facial masculinity is not exclusively a male ornament, as male ornaments are typically more sensitive to growth in males compared with females. Additionally, we measured immunocompetence via heterozygosity at the major histocompatibility complex (MHC), a widely-used genetic marker of immunity. We show that, while height is positively correlated with MHC heterozygosity, facial masculinity is not. Thus, facial masculinity does not reflect immunocompetence measured by MHC heterozygosity in humans. Overall, we find no support for the idea that facial masculinity is a condition-dependent male ornament that has evolved to indicate immunocompetence.

facial masculinity | MHC heterozygosity | sexual selection | immunocompetence handicap hypothesis | human evolution

The condition-dependent hypothesis is used to explain the evolution of male ornaments in nonhuman animals (1–5). According to this hypothesis, male ornaments (e.g., reindeer antlers and peacock trains), which grow to exaggerated proportions even though they might be detrimental to fitness (6, 7), are adaptations signaling the underlying physiological and genetic quality of the individual to females. Such traits are more sensitive to the overall growth of individuals and more variable than other traits (8–14). As growth itself is dependent on a variety of genetic and environmental factors, including immunocompetence, inbreeding, health status, and nutrient availability (15–19), slight variations in physiological and genetic quality among males are amplified to perceptible levels in sexual ornaments, making them reliable indicators of underlying health (8, 9, 13). The condition-dependent hypothesis has also been applied to humans to explain the evolution of secondary sexual characteristics, such as facial masculinity and deep voices (20–24). The apparent attraction of women to these traits, which appears to be heritable (25), is thought to be an evolutionary adaptation that helps women secure direct (e.g., investment of a healthy male) and indirect (e.g., "good genes" for their children) benefits (1, 3, 26, 27).

Among all of the factors placed under the umbrella of "condition," immunocompetence has received considerable attention. This is, in part, because of the supposed immunosuppressive effects of androgens (28, 29), which are involved in the development of secondary sexual traits in males (22). According to the immunocompetence handicap hypothesis (ICHH), androgens mediate the allocation of resources between the competing demands of fighting infections and the development of energetically "costly" sexual ornaments (30–41). Consequently, males with more effective immune systems may withstand higher androgen levels, and the accompanying immunosuppressive burden, and can "afford" more extravagant displays. If this were true, then secondary sexual characteristics could serve as reliable ("honest") indicators of the physiological and immunological quality of males (7, 35, 40, 42). Parts of the ICHH have found some support in humans (36, 43–45) and nonhuman animals [for review, see Roberts et al. (46)].

However, the evidence linking secondary sexual traits to the condition, immunological or otherwise, of human males is ambiguous and inconsistent across studies (44, 46–48). That androgens are immunosuppressive also does not appear to be well-supported (49). This has recently led many to question the applicability of the ICHH in humans, particularly with respect to facial masculinity (49–51). Some of the inconsistency has been attributed to methodological limitations, such as small sample

Significance

Facial masculinity has been considered a sexual ornament in humans, akin to peacock trains and stag antlers. Recently, studies have questioned the once-popular view that facial masculinity is a condition-dependent male ornament signaling immunocompetence (the immunocompetence handicap hypothesis). We sought to rigorously test these ideas using high-resolution phenotypic (3D facial images) and genetic data in the largest sample to date. We found no support for the immunocompetence handicap hypothesis of facial masculinity in humans. Our findings add to a growing body of evidence challenging a popular viewpoint in the field and highlight the need for a deeper understanding of the genetic and environmental factors underlying variation in facial masculinity and human sexual dimorphism more broadly.

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size and the use of measures of perceived masculinity and attractiveness, which are influenced by sociocultural factors that are difficult to control in observational studies (50). Another limitation that has received less attention is the lack of correction for ancestry and population structure, which can lead to spurious associations. Because of these issues, a rigorous study of the link between facial masculinity and immunocompetence and/or condition is needed.

In this study, we investigated the condition-dependent hypothesis and ICHH in humans with respect to facial masculinity. Working from theory and evidence from research on condition dependence of sexual ornaments in nonhuman animals (1–5, 8), we tested three hypotheses:

Hypothesis 1: Facial masculinity is a condition-dependent male ornament in humans. If this is true, then we expect facial masculinity to be (*i*) more strongly correlated with overall growth in males relative to females, and (*ii*) more variable in males compared with females.

Hypothesis 2: Immunocompetence is associated with overall growth in humans. If immunocompetence plays a role in condition-dependent expression of secondary sexual characteristics, then it should be correlated with overall growth in humans.

Hypothesis 3: Facial masculinity reflects immunocompetence in men. Males who show greater immunocompetence should exhibit more masculine faces than males with lower immunocompetence. In contrast, facial masculinity should be less sensitive to variation in immunocompetence in females.

To test our hypotheses, we used an objective measure of facial masculinity, calculated with high resolution 3D photographs in a large sample of persons of European ancestry. We used height as a proxy for overall growth and condition as height is known to be associated with health, income, nutrition, and exposure to disease and infection (15–17). We used individual heterozygosity at the major histocompatibility locus (MHC) as a measure of immunocompetence. The MHC locus, also known in humans as the HLA complex, is located on chromosome 6 and contains around 200 genes that are involved in immune function (52). Higher genetic diversity at the MHC enables the immune system to recognize a more diverse array of foreign antigens (52–54). As a result, the MHC has experienced balancing selection in both humans and nonhumans (52, 55–58). Therefore, heterozygosity at this locus serves as a useful proxy to measure immunocompetence. Finally, we considered the effects of body size on facial masculinity (allometry) in addition to other likely confounders, such as age, weight, genome-wide heterozygosity, and population structure.

Results

Variation in Facial Masculinity. We calculated high resolution facial masculinity (FM) for the faces of 1,233 males and females of European ancestry from 3D images using a scalar-projection approach, similar to that described in Valenzano et al. (59) (*SI Appendix*, Fig. S7). The 3D images were processed as described previously (60–62), allowing us to represent each face as a mesh of 7,150 points, or quasi-landmarks (QLs), each with *x*, *y*, and *z* coordinates. For every QL in the face, the signed difference between the coordinates of the average female and male faces represents the direction of sexual dimorphism in 3D space (*SI Appendix*, Fig. S7*A*). We defined FM for each of the 7,150 QLs (FM_{QL}) as the degree of change in a target face (X) along these vectors. Note that this measure includes both allometric (size-dependent) and nonallometric (size-independent) components of facial masculinity (63–65). We correct for allometry, when necessary, by residualizing FM on height or by including height as a covariate, which yielded identical results (*Materials and Methods* and *SI Appendix*, Fig. S8).

Fig. 1*A* shows a bimodal distribution of overall FM score (averaged across QLs—hereafter referred to as $FM_{overall}$) where values of 0 and 1 represent $FM_{overall}$ of the average female and male faces, respectively. The magnitude of sex difference in $FM_{overall}$ is comparable with that of height (Cohen's D of 1.98 compared with 2.10 for height). Expectedly, the magnitude of sex difference in the nonallometric component of $FM_{overall}$ was smaller (Cohen's D = 0.76). The brow ridge, cheekbones, and nose ridge showed the greatest degree of sexual dimorphism, in agreement with previous studies (Fig. 1*B*) (62, 63, 66).

Facial Masculinity Is Positively Correlated with Height in both Sexes. We tested the relationship between overall facial masculinity ($FM_{overall}$) and growth, using height as a predictor, with sex, age, weight, and genetic principal components (gPCs) 1 to 3 as covariates (*SI Appendix*, Fig. S11). $FM_{overall}$ is positively correlated with height in both sexes (Fig. 2*A* and Table 1; $T = 8.81$, $P = 4.17 \times 10^{-18}$), suggesting that taller people have more masculine faces than shorter people. Because variation in the size of the faces was removed before calculating facial masculinity (*Materials and Methods*), this correlation represents allometric effects of growth on sexual dimorphism in face shape, not size (64). The effect of height on facial masculinity appears to be concentrated around the orbital region, nasal bridge, cheeks, and the chin, with masculinity in these regions increasing with height (Fig. 2*B*), matching previous observations on the effects of allometry on faces (63, 64). It is interesting to note that the distribution of the effect of height on masculinity across the face appears to be different from the effect of sex (Fig. 2*B*), which is consistent with previous observations that facial masculinity is not exclusively a result of extended overall growth in males compared with females (63, 65–68).

The effect of height on $FM_{overall}$ is not significantly different between males and females ($\beta_{male} = 0.227$, $\beta_{female} = 0.299$, $Z\text{-}score_{diff} = -1.18$, $P = 0.120$), and a similar observation can be made for the regional effects of height on FM_{QL} (*SI Appendix*, Fig. S12). $FM_{overall}$ is also not significantly more variable in either sex (Levene's test $P = 0.37$ with adjustment for height and $P = 0.66$

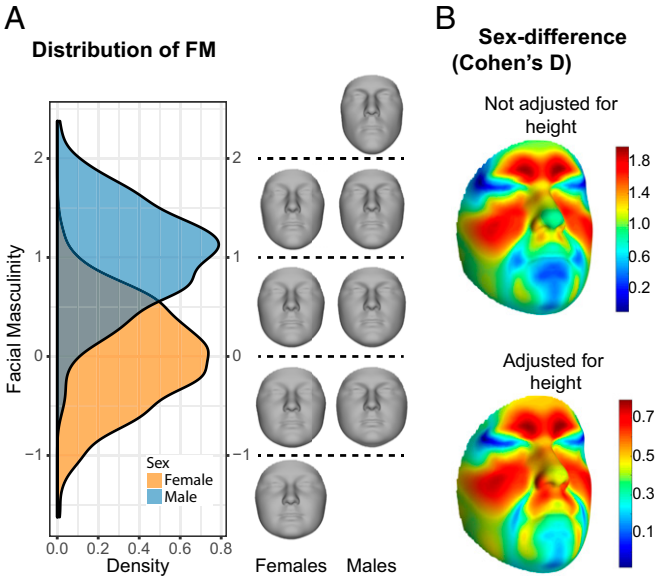

Fig. 1. Variation in facial masculinity and sexual dimorphism. (*A*) Density plot showing a bimodal distribution of facial masculinity. (*B*) Heat maps showing Cohen's D of dimorphism between average male and female faces before (*Top*) and after (*Bottom*) correction for allometry.

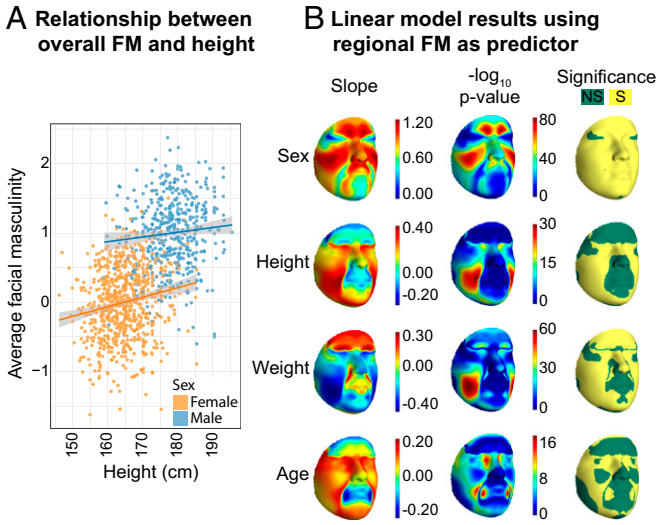

Fig. 2. Facial masculinity is positively correlated with height within and between the sexes. (*A*) Relationship between $FM_{overall}$ and height. (*B*) Results of linear model between FM_{QL} and height and other covariates. Regions in yellow are significant after Bonferroni correction for 7,150 QLs ($P < 0.05/7150 = 6.93 \times 10^{-6}$). *SI Appendix*, Fig. S11 shows results for all covariates.

without adjustment). Greater sensitivity to growth and higher variance in males relative to females are classic signatures of male ornaments (2, 5, 8, 69), an expectation that facial masculinity does not meet. It is interesting to note that FM varies significantly along gPCs 1 and 2, suggesting that the patterns of sexual dimorphism and facial masculinity vary across populations, even within Europe (Table 1). This not only highlights the need to correct for population structure in future studies but also calls for a detailed exploration of the variation in facial shape across populations.

Height Is Positively Correlated with Immunocompetence. We fit a linear model between height and MHC heterozygosity, while correcting for genome-wide heterozygosity, sex, age, and gPCs 1 to 3. MHC heterozygosity showed a positive correlation with height (Table 2; $T = 3.18$, $P = 0.0015$), indicating that individuals who are more heterozygous at the MHC locus tend to be taller than people who are less heterozygous. This relationship is not driven by genome-wide heterozygosity as the latter is not significantly associated with height (Table 2; $T = -0.36$, $P = 0.72$). Also note that height varies significantly along gPC1 (Table 2), which is consistent with the clinal variation in stature observed within Europe (18, 70).

Facial Masculinity Is Not Correlated with Immunocompetence. MHC heterozygosity is not significantly correlated with overall facial masculinity regardless of whether height is included in the model ($T = -0.038$, $P = 0.970$) or not ($T = 0.586$, $P = 0.558$). Thus, neither the allometric nor the nonallometric component of facial masculinity is informative about MHC heterozygosity. MHC heterozygosity is also not significantly correlated with regional measures of facial masculinity (FM_{QL}; *SI Appendix*, Fig. S13). Furthermore, there is no correlation between facial masculinity and genome-wide heterozygosity (height included as covariate: $T = 0.02$, $P = 0.986$; height not included as covariate: $T = 0.65$, $P = 0.516$). Finally, there is no difference in the effect of MHC heterozygosity on facial masculinity between males and females ($\beta_{male} = 0.035$, $\beta_{female} = -0.024$, $Z\text{-}score_{diff} = 0.32$, $P = 0.375$; *SI Appendix*, Fig. S14).

Discussion

Condition-dependent male ornaments tend to be highly variable and more sensitive to variation in growth among males (i.e., exhibit greater allometric effects in males compared with females) (8, 10, 13, 14). Facial masculinity does not meet these expectations as neither the allometric nor the nonallometric component of facial masculinity is more variable in males compared with females, and the allometric effects of growth on facial masculinity are similar across the sexes.

Because diversity at the MHC locus is important for antigen recognition and presentation and the locus is known to be under balancing selection in many species, including humans, heterozygosity at this locus is an important marker of immunocompetence. This is supported by our finding that people who are more heterozygous at the MHC locus are taller, on average, than people who are less heterozygous, suggesting that variation at the MHC locus might be important for growth. We find that facial masculinity is not significantly associated with MHC heterozygosity, either in males or females. Our results do not support the hypothesis that facial masculinity is an indicator of immunocompetence. However, we cannot rule out the effect of other measures of immunocompetence such as non-MHC genes and antibody titers. We also find no support for the contention that FM indicates heterozygosity across the genome generally, something that has also been hypothesized previously (3, 71).

Altogether, our findings add to the growing number of studies questioning some of the evolutionary explanations behind female and male perceptions of facial masculinity (50, 51) and whether masculinity should be regarded as a condition-dependent male ornament in humans. Nevertheless, there are many questions related to facial sexual dimorphism and masculinity in humans that need to be addressed from an evolutionary standpoint. Humans show intermediate levels of allometric cranial sexual dimorphism among extant hominids (65), and we do not know whether this degree of sexual dimorphism is new to humans since their divergence from other hominins and apes. Did some aspects of facial masculinity evolve as a mechanism to intimidate rival males (72–74), or do they represent vestigial traits that have decreased over time as a result of self-domestication (75–77)? We also do not know how facial sexual dimorphism varies across human populations although we suspect that it does so considerably, both in degree and pattern, given that our results show that it varies significantly across Europe. Is genetic drift sufficient to explain these patterns? If not, can these patterns be explained by differences in perceptions of beauty and social status across populations? It is important to tackle these questions by careful comparison of the degree and pattern of sexual dimorphism within and across populations, as well as how sexual dimorphism is perceived cross-culturally.

Equally important is the need to fill gaps in our knowledge of the proximate causes underlying sexual dimorphism and facial

Table 1. Results of linear model between $FM_{overall}$ and height with covariates

Predictor	Slope (β)	95% CI	*T* statistic	*P* value
Sex	1.240	1.126, 1.353	21.46	**5.55×10^{-87}**
Height	0.260	0.201, 0.319	8.81	**4.17×10^{-18}**
Age	0.109	0.070, 0.148	5.60	**2.69×10^{-08}**
Weight	−0.221	−0.266, −0.176	−9.64	**3.07×10^{-21}**
gPC1	−0.083	−0.122, −0.044	−4.19	**2.94×10^{-05}**
gPC2	−0.063	−0.100, −0.026	−3.25	**1.78×10^{-03}**
gPC3	−0.052	−0.089, −0.015	−2.68	7.55×10^{-03}

Slopes are standardized regression coefficients. Bonferroni cutoff for significance is 0.05/7 = 0.007. *P* values smaller than the Bonferroni cutoff are shown in boldface type.

Table 2. Results of linear model between height and MHC heterozygosity

Predictor	Slope (β)	95% CI	T statistic	P value
MHC het.	0.063	0.014, 0.112	3.18	$\mathbf{1.54 \times 10^{-03}}$
Genome-wide het.	−0.007	−0.046, 0.032	−0.36	0.72
Sex	1.452	1.374, 1.530	36.20	$\mathbf{9.43 \times 10^{-196}}$
Age	0.022	−0.017, 0.061	1.11	0.266
gPC1	0.122	0.083, 0.161	6.11	$\mathbf{1.36 \times 10^{-09}}$
gPC2	0.051	0.012, 0.091	2.54	1.14×10^{-02}
gPC3	0.014	−0.025, 0.053	0.70	0.49

Slopes are standardized regression coefficients. Bonferroni cutoff for significance is 0.05/7 = 0.007. P values smaller than the Bonferroni cutoff are shown in boldface type. het., heterozygosity.

masculinity. We know that differences in facial shape exist between male and female children as young as 3 y old (66, 68, 78) and are likely defined, in part, by the intrauterine environment during gestation (79–81). This dimorphism increases dramatically at the onset of puberty, implicating sex hormones and other endocrine processes underlying general growth during this period (66, 68, 82–85). These observations suggest that facial masculinity may arise because of extended overall growth and higher circulating androgen levels in pubertal males (66, 84, 86). However, sex differences in face shape are not merely developmental byproducts of extended overall growth in males as we and others have shown that sex has a significant effect on facial shape even after adjusting for body size (63, 65–68). Variation in facial masculinity also cannot be attributed solely to differences in circulating androgens during puberty. This is clear from the observation that, despite the fact that males exhibit higher mean and variance in androgen levels compared with females (87), they are not more variable in terms of facial masculinity. In fact, a recent report shows that the heritability of facial masculinity is similar between males and females, and the correlation between facial masculinity of same-sex siblings is similar to that of opposite-sex siblings (88). These results are indicative of a shared genetic architecture underlying facial masculinity in males and females and further serve to deemphasize the idea that facial masculinity is a male-specific ornament. It will be important to explore the effects of other hormones (e.g., estrogen and estradiol) and sex-chromosomal genes (89–91), as well as the timing of these effects. These questions are fundamental for cultivating a more mechanistic understanding of the development of sexual dimorphism, which, in turn, will lead to a better understanding of the role of sexual selection in human evolution.

Materials and Methods

Participant Recruitment. Study participants were recruited with written informed consent in the United States through the Anthropology, DNA, and the Appearance and Perceptions of Traits (ADAPT) Study. All aspects of the study were approved by the Pennsylvania State University Institutional Review Board (no. 44929 and 45727). The 3D images were taken using the 3dMD Face system (3dMD). Height and weight were measured using an Accustat stadiometer (Genentech) and clinical scale (Tanita). Genotyping was conducted by 23andMe (23andMe) on the v4 genome-wide SNP array. After filtering out SNPs with more than 10% missing genotypes, this array comprised 567,787 SNPs.

Data Curation. From the 2,721 participants with faces and genotype data, we removed individuals with missing covariate data, misclassified sex information, and individuals with more than 10% missing genotypes. We further restricted the analysis to unrelated individuals between 18 and 30 y of age (to reduce the effects of aging). Relatives were identified as pairs of individuals with an identity-by-state (IBS) value of at least 0.8, after which one of each pair was removed, resulting in a set of 1,921 unrelated individuals (*SI Appendix*, Fig. S1).

Ancestry and Population Structure. We selected people of European ancestry as they comprised the largest sample in our dataset. To do so, we merged the genotype data from our sample (n = 1,921) with genotypes from the 1,000 Genomes Project dataset (n = 2,503) (92). Before the merge, we removed SNPs that did not intersect between the two datasets, palindromic (A/T, G/C) SNPs, and SNPs that did not meet standard quality-control criteria (*SI Appendix*, Fig. S1). SNPs were further pruned for linkage disequilibrium (LD) with a window size of 50 SNPs, a step size of 5 SNPs, and a variance inflation factor threshold of 2 using PLINK 1.9 (93, 94), resulting in 201,042 SNPs. Genetic ancestry was inferred using an unsupervised clustering scheme in ADMIXTURE, with K ranging from 2 to 16 (*SI Appendix*, Figs. S2 and S4) (95). We selected results from K = 6 as this value had a low cross-validation error (*SI Appendix*, Fig. S3) and showed separation based on continental ancestry (*SI Appendix*, Fig. S2). Then 1,249 individuals of primarily European ancestry were identified based on ADMIXTURE output by comparison with European samples from the 1,000 Genomes Project (*SI Appendix*, Fig. S4). We carried out principal components analysis on the genotypes of this subset and removed 16 outliers using the smartpca program in Eigensoft (*SI Appendix*, Fig. S5) (96, 97), leading to a sample size of 1,233 individuals. The first three genetic PCs (gPCs) were used as covariates to correct for population structure (*SI Appendix*, Fig. S5), which is minimal in our dataset beyond the first three gPCs (*SI Appendix*, Fig. S6).

Processing 3D Photographs. High-resolution 3D images were "cleaned" to remove hair, ears, and disassociated polygons. Five positioning landmarks were placed (two on the inner corner of the eyes, two on the outer corners of the mouth, and one on the tip of the nose) to establish facial orientation. An anthropometric mask comprised of 10,000 quasi-landmarks (QLs), which was later trimmed to 7,150 QLs, was nonrigidly mapped onto all 3D surfaces such that each QL was spatially homologous across individuals (60–62). Thus, every face could be represented by a configuration of 7,150 QLs, each with three coordinates (x, y, and z). For each face, a mirror image was created by changing the sign of the x coordinates following (98), which was mapped with QLs in the same way as the original, nonreflected face. A generalized Procrustes superimposition (99) of both the original and reflected images together was performed to eliminate differences in position, orientation, and scale. The original and reflected images were then averaged to create a symmetric facial shape (62).

Calculating Facial Masculinity. We define facial masculinity as the degree of change in the direction from an average female face to an average male face. Conversely, facial femininity is the degree of change in the opposite direction. We calculated facial masculinity (FM) per quasi-landmark (QL) for every face, using a scalar-projection approach (*SI Appendix*, Fig. S7) (59, 63, 100, 101). First, we generated female and male consensus faces from the sample by averaging the QL configurations across all females and all males, respectively. For every QL on the face, the signed difference between the coordinates of the male and female consensus faces was a 3D vector $\overrightarrow{V_{FM}}$ that represents the direction of sexual dimorphism in 3D space (*SI Appendix*, Fig. S7*A*). The goal was to calculate the degree of change in each QL of a target face X along these vectors (i.e., one for each of the 7,150 QLs), which is the FM per QL (FM_{QL}). This could be done by computing the scalar projection of $\overrightarrow{V_{FX}}$, the difference between X and the female consensus face, onto $\overrightarrow{V_{FM}}$ (*SI Appendix*, Fig. S7*C*):

$$\text{Facial masculinity per QL}\,(FM_{QL}) = \frac{\overrightarrow{V_{FM}} \cdot \overrightarrow{V_{FX}}}{\left|\overrightarrow{V_{FM}}\right|}.$$

Note that this measure represents both allometric and nonallometric components of FM. We corrected for the effects of allometry where necessary by including height as a covariate in the regression models. We show in *SI Appendix* that this is equivalent to other approaches, such as residualizing the original shape coordinates on height before constructing the male and female consensus faces and calculating FM (*SI Appendix*, Fig. S8) (63).

Genomic and MHC Heterozygosity. We defined individual heterozygosity as the proportion of heterozygous SNPs in a region. Genome-wide heterozygosity was calculated from a total of 192,417 LD-pruned, autosomal SNPs. To measure MHC heterozygosity, we obtained a list of 195 SNPs tagging haplotype variation for the classical HLA genes in Europeans (102). We used 114 of these SNPs, the subset for which our samples were genotyped (*SI Appendix*, Fig. S9), to calculate MHC heterozygosity. These SNPs captured most of the HLA alleles (102) and the heterozygosity calculated

using the subset of 114 SNPs was highly correlated with heterozygosity calculated using a larger subset ($n = 154$ SNPs) for which the sample of Europeans available in the 1000 Genomes Project dataset were genotyped (*SI Appendix*, Fig. S10) (92).

Data Availability. The informed consent with which the data were collected does not allow for dissemination of identifiable data to persons not listed as researchers on the IRB protocol. Thus, the raw genotype data and 3D images cannot be made publicly available. In the interest of reproducibility, we have provided deidentified overall facial masculinity measures as well as age, sex, weight, height, ancestry, genetic PCs, and MHC and genome-wide heterozygosity, from which all results presented in this manuscript can be reproduced (Dataset S1). In addition, we provide high-density facial masculinity maps: i.e., facial masculinity calculated for every quasi-landmark (FM_{QL}) for all 1,233 individuals used in the analyses (Dataset S2). Lastly, we provide all protocols and scripts used to process the genetic data and perform the analyses as well as a tutorial showing how FM_{QL} can be used and visualized. This resource will allow other researchers to study variation in facial masculinity with high resolution in a large sample. All of the above are available on the following GitHub repository: https://github.com/Arslan-Zaidi/Facial_masculinity_MHC.

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