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## **Title**

**Genetic variants underlying differences in facial morphology in East Asian and European populations**

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## **Abstract**

**Facial morphology, a conspicuous feature of human appearance, is highly heritable. Previous studies on the genetic basis of facial morphology were mainly performed in European-ancestry cohorts (EUR). Applying a data-driven phenotyping and multivariate genome-wide scanning protocol to a large collection of 3D facial images of individuals with East Asian ancestry (EAS), we identified 244 variants in 166 loci (62 novel) associated with typical-range facial variation. A newly proposed polygenic shape analysis indicates that the effects of the variants on facial shape in EAS can be generalized to EUR. Based on this, we further identified 13 variants related to differences between facial shape in EUR and EAS populations. Evolutionary analyses suggest that the difference in nose shape in EUR and EAS populations is caused by a directional selection, mainly due to a local adaptation in Europeans. Our results illustrate the underlying genetic basis for facial differences across populations.**

1 **Main Text**

2 Facial morphology has substantial variations at the individual and population  
3 level. Multiple studies show significant differences in craniofacial morphology  
4 across people from different geographic regions<sup>1-18</sup>. For example, individuals of  
5 European ancestries (EUR) have a more protruding nose and brow ridges than  
6 those of East Asian ancestries (EAS)<sup>19</sup>. Such differences must have a strong  
7 genetic basis, which remains unknown, largely due to the low number of studies  
8 in performed in EAS populations compared to EUR. Previous genetic studies  
9 collectively reported about 219 loci associated with facial morphology in EUR  
10 populations, but only 24 were reported in EAS or Eurasian-ancestry populations,  
11 about 20 in Latin American-ancestry populations, and four in African-ancestry  
12 populations (Supplementary Table 1)<sup>3-5,9,12-14,16,17</sup>. Large-scale studies in EAS  
13 populations and other non-European populations are much needed to provide  
14 a complete architecture of the genetic basis of facial morphology, particularly  
15 the observable differences across populations.

16 Here we performed a genome-wide association study (GWAS) based on a large  
17 collection of 3D facial images from the Han Chinese population. Using a data-  
18 driven phenotyping approach, we identified hundreds of associated variants<sup>10,18</sup>.  
19 In addition, we identified specific variants distinguishing facial appearance  
20 between EUR and EAS populations. We further provided evidence that those  
21 population-based facial differences, especially for nose shape, were under  
22 selection. A schematic overview of our study design can be found in Extended  
23 Data Figure 1.

24

25 **GWAS on facial phenotypes discovered 244 leading variants.**

26 To study facial variation from a global to local scale, we used 3D facial surface  
27 scans from a large-scale EAS population (Methods, Supplementary Table 2) in  
28 a discovery ( $n = 6,968$ ) and replication cohort ( $n = 2,706$ ), and subsequently  
29 combined them in a meta-analysis. A semi-supervised phenotyping procedure  
30 defined 63 hierarchically arranged facial segments using the discovery cohort  
31 (Methods). Next, we performed a canonical correlation analysis (CCA) based  
32 GWAS on each facial segment's group of principal components (Methods).  
33 Subsequently, we identified 50 independent tests using parallel analysis and  
34 permutation test (Methods, Supplementary Note)<sup>18,20,21</sup>. Thus, besides  
35 conventional genome-wide significant threshold ( $P = 5 \times 10^{-8}$ ), we set a stricter  
36 study-wide significant threshold to  $P = 9.8 \times 10^{-10}$  ( $P = 5 \times 10^{-8} / 51.41$ ) after  
37 Bonferroni correction for multiple testing. In the discovery datasets, we  
38 identified 153 genome-wide significant variants ( $P < 5 \times 10^{-8}$ , minor allele  
39 frequency (MAF)  $> 0.05$ ) in 124 loci after condition analysis and peak selection  
40 (Methods). In the replication dataset, 119 out of 153 (77.8%) variants were  
41 replicated at nominal significance ( $p < 0.05$ ), 118 (77.1%) were replicated at  
42  $FDR < 0.05$ , 75 (49.0%) were replicated at a Bonferroni-corrected significance  
43 ( $P < 3.28 \times 10^{-4}$ ). For the 84 variants that passed the study-wide significance ( $P$   
44  $< 9.8 \times 10^{-10}$ ), 80 out of 84 (95.2%) were replicated at nominal significance  
45 ( $p < 0.05$ ) and  $FDR < 0.05$ , 65 (77.4%) were replicated at a Bonferroni-corrected  
46 significance ( $P < 5.95 \times 10^{-4}$ ). To increase statistical power, we performed a  
47 meta-analysis using Stouffer's method to combine the P-values obtained from  
48 the discovery and replication cohort<sup>18,22</sup>. As a result, we identified 244  
49 independent variants in 166 loci under the genome-wide threshold associated

50 with normal range facial variation (Fig. 1a; Supplementary Table 3) where 151  
51 variants in 106 loci were study-wide significant. According to the anatomical  
52 structure, we classified the 244 genome-wide significant variants in ten facial  
53 regions (Supplementary Note), including forehead, glabella, eye, tempora,  
54 zygoma, nose, maxillary, upper mouth, lower mouth, and mandible (Fig. 1b;  
55 Supplementary Table 4). The nose was the feature associated with the most  
56 variants (107) out of the ten regions. The numbers of variants associated with  
57 the other nine regions were: glabella (35), upper mouth (32), eye (29), zygoma  
58 (28), maxillary (25), mandible (20), forehead (16), lower mouth (13) and  
59 tempora (12). In addition, we calculated the genome-wide heritability of each  
60 3D facial segments at each level. The 3D facial segments' heritability ranged  
61 from 7.47% to 52.3%. As we expected, the nose segments were also among  
62 the most heritable area (42.17% to 46.86%, Supplementary Fig. 1).

63 We considered a variant as novel when it was not in linkage disequilibrium (LD,  
64  $r^2 < 0.1$ ) with previously reported variants in facial GWASs ( $P < 5 \times 10^{-8}$ ,  
65 Supplementary Table 1) in any East Asian (EAS), European (EUR), and African  
66 (AFR) populations in the 1000 Genomes Project phase 3 (1000GP, Methods)<sup>23</sup>.  
67 As such, 130 of the 244 leading variants under the genome-wide threshold were  
68 novel, while 65 of the 151 under the study-wide threshold were novel  
69 (Supplementary Table 3). We considered a genetic locus as novel when it did  
70 not overlap with previously reported genomic loci associated with facial  
71 variation. As such, 62 out of 166 loci were novel.

72 We used FUMA and GREAT to annotate the leading variants<sup>24,25</sup>. As a result,  
73 we identified 206 candidate genes potentially associated with facial variation,  
74 among which 100 genes were not reported in previous facial GWASs

75 (Supplementary Table 3). We found that the genes associated with the leading  
76 variants were highly enriched in biological processes of skeletal system  
77 development and morphogenesis (Extended Data Fig. 2a). Moreover, the  
78 epigenome and transcriptome datasets showed that the leading variants were  
79 mainly enriched for enhancers in craniofacial tissues. Compared with the  
80 ectoderm at the later stage of fetal development, candidate genes were  
81 significantly highly expressed in the mesenchyme (Extended Data Fig. 2b-c),  
82 consistent with our expectation and previous studies<sup>18,26-30</sup>.

83 We investigated associations ( $P < 1 \times 10^{-5}$ ) with other complex traits for the 244  
84 variants through PhenoScanner, a web-based GWAS repository (Methods)<sup>31</sup>.

85 We found that the traits sharing high genetic components with facial shapes  
86 mainly involved physical measurements, body composition and hair  
87 morphology (Supplementary Table 5). The highest co-association was the risk  
88 of atrial fibrillation (*PITX2*, rs6843082,  $P = 3.0 \times 10^{-155}$ ). This variant has been  
89 reported to be associated with cardioembolic stroke and ischemic stroke,  
90 suggesting that facial features might be a biomarker of cardiovascular disease.

91

### 92 **Characteristics of specific variants in EAS and EUR**

93 By comparing the 244 leading variants identified in our study with the 203  
94 leading variants reported in a recently published EUR study using similar  
95 phenotyping and analysis framework, 89 variants were shared in both studies  
96 (Methods)<sup>18</sup>. The remaining 155 and 114 variants in the EAS and EUR studies  
97 respectively were different (Fig. 2a; Supplementary Table 6). Therefore, we  
98 defined three different groups: 89 shared variants, 155 EAS-specific variants,  
99 and 114 EUR-specific variants.



100 To understand these shared and population-specific variants' characteristics,  
101 we examined their allele frequencies in EAS and EUR populations based on  
102 the 1000GP<sup>23</sup>. Comparison of cross-population minor allele frequency (MAF)  
103 showed that the groups of population-specific variants had higher MAF in their  
104 respective population ( $P_{EAS-specific} = 9.0 \times 10^{-12}$ ,  $P_{EUR-specific} = 8.1 \times 10^{-9}$ ). In  
105 contrast, the shared variants had no difference of MAF between the two  
106 populations ( $P_{shared} = 0.1$ ) (Fig. 2b-e). These results suggest that higher MAFs  
107 may increase the statistical power to detect the variants associated with facial  
108 variation within the respective populations. Moreover, 77.5% (69 out of 89) of  
109 the shared variants passed the study-wide significance threshold while only  
110 54.2% (84 out of 155) EAS-specific variants and 55.2% (63 out of 114) EUR-  
111 specific variants passed the study-wide significance threshold (chisq.test  $P_{EAS}$   
112  $= 3.2 \times 10^{-4}$ ;  $P_{EUR} = 4.8 \times 10^{-6}$ ).

113 To explore potential biological functional differences between the shared and  
114 population-specific variants, we used Metascape to compare the differences of  
115 enriched terms for their annotated genes<sup>32</sup>. We found that EAS-specific, EUR-  
116 specific, and shared genes were all associated with top terms that were  
117 previously reported in association with craniofacial variation (Fig. 2f). Moreover,  
118 we observed a considerable number of functional overlaps among the three  
119 groups (Fig. 2g). These results indicate that the associated genes across  
120 populations share substantially similar biological processes. We next compared  
121 epigenetic regulation patterns of the shared and population-specific variants in  
122 various cell-types or tissues. The shared and population-specific variants were  
123 all enriched for enhancers in craniofacial tissues (Fig. 2h), again indicating that  
124 the potential functions of facial variants across populations are analogous.

125 As expected, we found that the population-specific variants had a significantly  
126 higher Wright's fixation index ( $F_{ST}$ ) than random variants from a genome-wide  
127 background both in EAS and EUR populations ( $P_{EAS-specific} = 2.42 \times 10^{-10}$ ;  $P_{EUR-}$   
128  $specific = 0.0063$ , Extended Data Fig. 3), while the shared variants had no  
129 significant difference compared with random variants ( $P_{EAS-shared} = 0.599$ )<sup>33,34</sup>.  
130 The same result applied to the Cross Population Extended Haplotype  
131 Homozygosity (XP-EHH) analysis using REHH2 in these two populations ( $P_{EAS-}$   
132  $specific = 0.0078$ ;  $P_{EUR-specific} = 0.038$ ;  $P_{EAS-shared} = 0.449$ )<sup>35,36</sup>. These results  
133 suggest that facial variation across populations could be attributed to random  
134 drift and natural selection. The population-specific variants explained a larger  
135 proportion of natural selection while the shared variants may mainly explain  
136 random drift influencing facial variation.

137 In summary, the population-specific variants found in EAS and EUR were  
138 identified due to cross-population MAF differences and subtle effect sizes.  
139 Nonetheless, similar biological processes underlying facial variation were  
140 shared across populations.

141

#### 142 **Polygenic shape analysis generalizes results from EAS to EUR**

143 To explore the genetic basis of EAS facial shape and the genetic factors  
144 contributing to the difference in facial shape between EAS and EUR, we first  
145 investigated whether the association results of the leading variants in our EAS  
146 study could be generalized to EUR.

147 We introduced a novel polygenic shape analysis to investigate whether the  
148 differential accumulated genetic effects between the two populations of the  
149 leading variants is in line with the actual population facial differences. Similar to

150 the classic polygenic score analysis, we defined the Polygenic Shape (PS) for  
151 an individual as the sum of the number of effect risk alleles weighted by risk  
152 allele effect size of all the leading variants<sup>37</sup>. Similarly, the Polygenic Population  
153 Shape (PPS) is the average polygenic shape for a given population (Methods).  
154 Using data of EUR ( $n = 404$ ) and EAS ( $n = 208$ ) individuals from 1000GP, we  
155 calculated the PPS of the two populations for the whole face and ten anatomical  
156 facial regions.

157 To visualize and compare this effect to the true population facial shape average  
158 for each facial region, we constructed EUR and EAS PPS-derived faces by  
159 adding and subtracting, respectively,  $(PPS_{EUR} - PPS_{EAS})/2$  to a population  
160 neutral average face, which was constructed as the average of the EUR and  
161 EAS population average shapes (Methods). We used 3D facial scans of EAS  
162 and EUR individuals to calculate each population's average face and therefore  
163 generated EUR and EAS average faces. Compared with the average face of  
164 EUR, we found that EAS had more protrusion in the cheek; more concavity in  
165 the forehead, glabella, nose, and mandible (Fig. 3a-i), which were consistent  
166 with a previous study<sup>19</sup>. Interestingly, when we amplified the differentiated  
167 accumulated genetic effects five times, the PPS-derived faces looked very  
168 similar to EAS and EUR's actual average face (Fig. 3a-ii). The EUR and EAS  
169 PPS-derived faces showed similar facial variation to the ground truth, especially  
170 in the glabella and nose region (Fig. 3a-ii).

171 To test the generalization of the association results from our study to EUR, we  
172 compared the EUR and EAS PPS-derived faces of the whole face using all the  
173 244 leading variants with the PPS of the whole face using 244 variants  
174 randomly chosen from the genome. The EAS and EUR PPS-derived faces

175 using the leading variants were significantly more similar to the true population  
176 average faces than that using random variants, either measured by Euclidean  
177 distance ( $P_{dis} = 0.007$ ) or cosine similarity ( $P_{EUR} = 0.004$ ;  $P_{EAS} = 0.005$ ; Fig. 3b).  
178 Moreover, we calculated individual facial polygenic shape for EUR and EAS  
179 individuals from 1000GP and measured their East Asian ancestry facial  
180 appearance (EAS-FA), defined as the projected length of the individual's  
181 polygenic shape onto the explicit EAS-EUR shape difference (Methods,  
182 Supplementary Fig. 2). The EAS-FA of both EUR and EAS groups were  
183 significantly separated, with EUR individuals closer to the EUR face and EAS  
184 individuals closer to the EAS face (t.test  $P < 2.2 \times 10^{-16}$ , Fig. 3c).

185 We also performed the same analyses locally for ten anatomical facial regions.  
186 The EAS and EUR PPS-derived shapes using the leading variants were  
187 significantly more similar to the population average shapes than those using  
188 random variants, involving the upper mouth, nose, maxillary, glabella, eye,  
189 tempora and zygoma in all the three measurements of similarity including  
190 cosine similarity, Euclidean distance, and EAS-FA (Extended Data Fig. 4a).  
191 Notably, the nasal region performed the best among the ten anatomical regions  
192 ( $P_{dis} = 4.8 \times 10^{-33}$ ;  $P_{EUR} = 3.7 \times 10^{-37}$ ;  $P_{EAS} = 3.4 \times 10^{-37}$ ; Fig. 3d-f). However, we  
193 obtained non-significant results for the mandible, forehead, and lower mouth  
194 (Extended Data Fig. 5). These results indicate that the PPS constructs  
195 morphological variations in most facial regions between EUR and EAS. Our  
196 results demonstrates that the PPS-derived faces using the leading variants are  
197 similar, both visually and statistically, to the true population average shapes at  
198 global and local scales, suggesting that the facial shape effects identified from  
199 the EAS study generalize well to EUR populations.

200

201 **Variants contribute to EAS facial appearance (EAS-FA)**

202 Among the leading variants, we aimed to find those variants that make EAS  
203 have more East Asian facial features, in other words, those variants that  
204 increase East Asian facial appearance (EAS-FA). We defined the contribution  
205 of a variant to EAS-FA as the effect allele frequency weighted projected length  
206 of its effect size vector onto the EAS-EUR shape difference (Methods,  
207 Supplementary Fig. 3, and Supplementary Table 7). A variant with a positive  
208 EAS-FA contribution may cause the EAS population's facial morphology to  
209 increase EAS-FA. In contrast, a variant with a negative EAS-FA contribution  
210 may cause the EAS population to increase EUR facial appearance. In brief, the  
211 variants with large positive EAS-FA are of our interest. In each facial region, we  
212 constructed a distribution of the variant's contribution to EAS-FA using 244  
213 leading variants. We further calculated whether each leading variant has  
214 significantly higher contribution to EAS-FA than the distribution after Bonferroni  
215 correction ( $P < 0.005$ , Extended Data Fig. 6, and Supplementary Table 8). As  
216 a result, 13 variants that passed filtering were considered to increase EAS-FA  
217 (Table1; Supplementary Fig. 4).

218 The 13 variants had a higher  $F_{ST}$  than the other variants (t.test  $P < 1.0 \times 10^{-16}$ ),  
219 indicating that they have significant allele frequency differences between EUR  
220 and EAS populations. The Population Branch Statistics (PBS) values of the  
221 variants were significantly higher in EAS ( $P < 1.0 \times 10^{-16}$ ) relative to EUR and  
222 YRI (Yoruba), but not in EUR ( $P = 0.188$ ) relative to EAS and YRI, which  
223 suggests that these variants may be under selection in EAS<sup>38</sup>. Thus, these  
224 variants potentially contribute to the morphological differences between EUR

225 and EAS. Most of the EAS-FA variants might be standing genetic variations, as  
226 the alternative allele frequency was relatively high, given the evolutionary time,  
227 as shown in Table 1. Notably, six variants had  $F_{ST}$  above 0.5 between EUR and  
228 EAS. Furthermore, three of these variants affected the glabella segments, and  
229 two affected the nasal region. This result suggests that local adaptation might  
230 play a role in forming facial variation between EAS and EUR. Among the 13  
231 variants, six were reported to be associated with facial shape variation. Well-  
232 known facial genes such as *EDAR*, *TBX15* and *MRPS22* were associated with  
233 craniofacial morphology in many studies<sup>3,4,6,10,13,15,16,18,39-43</sup>. Our study showed  
234 a variant in an intron of *TBX15* (rs10923710, NC\_000001.10:g.119502774G>T)  
235 contributing to maxillary and tempora shape in EAS, consistent with the  
236 observation that this locus had multiple spatial effects on the face<sup>18</sup>. The  
237 rs12632544 (NC\_000003.11:g.138946868T>A) is an intergenic variant near  
238 *MRPS22* on chromosome 3q23. It is in LD ( $r^2 = 0.932$ ) with rs12633011, which  
239 was reported associated with morphology of eyes in a previous EAS study<sup>16</sup>.  
240 *MRPS22* was reported to be associated with human earlobe size and a mouse  
241 skeleton phenotype<sup>3,44,45</sup>. A reanalysis of a GWAS study on cranioskeletal  
242 variation in outbred mice showed that variants in the region of chromosome 9,  
243 overlapping with *Mrps22*, were significantly associated with craniofacial  
244 variation (FDR < 5%, Supplementary Fig. 5). These variants were associated  
245 with the protrusion of the maxillary bone, the shrinkage of the eye and malar  
246 bone. In our study, rs12632544 contributes to EAS-FA in the glabella, eye and  
247 tempora (Extended Data Fig. 4b), in line with the result in the outbred mice. We  
248 also identified seven novel variants contributing to EAS-FA, five out of which  
249 are located closely to novel genes. Some of these have been reported in the

250 context of craniofacial dysmorphology. For instance, the rs6669519  
251 (NC\_000001.10:g.75584009T>A), which contributes to the shape of glabella,  
252 is an intergenic variant near *LHX8* on chromosome 1p31.1. *LHX8* (LIM  
253 Homeobox 8) was reported to be associated with cleft palate, forebrain neuron  
254 development and differentiation<sup>46,47</sup>. The rs12473319  
255 (NC\_000002.11:g.232880971G>C), an intronic variant of the *DIS3L2* gene,  
256 showed association with EAS-FA in glabella (Extended Data Fig. 4c). *DIS3L2*  
257 was found to affect a skeleton phenotype in a mouse genome study<sup>45,48</sup>. In  
258 addition, *DIS3L2* is a candidate gene for the Perlman syndrome, characterized  
259 by craniofacial abnormalities. Moreover, the frequency of the derived C allele is  
260 higher in EAS (47.8%) than EUR (2.2%). The estimated allele age of this variant  
261 is about 7,875 years old (Table 1), when EAS and EUR were already separated,  
262 suggesting that this variant arose in East Asian populations<sup>49</sup>.

263 Interestingly, there are four independent variants associated with nose  
264 morphology in the *SOX9* locus in our EAS GWAS (Extended Data Fig. 7).  
265 Although *SOX9* is a well-known gene contributing to the variation in nose shape,  
266 rs8068343 (NC\_000017.10:g.69447706T>C) is a novel variant that affects  
267 nose shape differences between populations<sup>7,9,10</sup>. In contrast, the other three  
268 variants may affect nose shape within populations. Compared with the variants  
269 previously identified near *SOX9*, the reference (T) allele of rs8068343 has a  
270 lower frequency in EUR (0.04) than in EAS (0.46). Moreover, this variant had a  
271 significantly higher  $F_{ST}$  (0.528) between EUR and EAS than any other variants,  
272 and the Integrated Haplotype Score (iHS) of this variant was also significantly  
273 higher in CHB (Han Chinese, 2.55)<sup>50</sup>. These results indicate that this variant  
274 contributes to EUR-EAS nose shape difference.

275

## 276 **Nose-associated variants are under positive selection**

277 To assess whether the variation of facial morphology in EAS and EUR  
278 populations is due to natural selection or random drift, we conducted several  
279 selection analyses of the leading variants discovered here. The  $F_{ST}$  enrichment  
280 test showed that regions of the whole face and nose had a significantly higher  
281  $F_{ST}$  than random variants after Bonferroni correction ( $P_{whole\ face} = 8.22 \times 10^{-7}$ ,  
282  $P_{nose} = 1.00 \times 10^{-4}$ , Supplementary Table 9; Fig. 4a), indicating that facial  
283 morphology has been under natural selection in EAS and EUR, especially in  
284 the nose region<sup>51</sup>. XP-EHH enrichment analysis showed a consistent result  
285 ( $P_{whole\ face} = 3.78 \times 10^{-3}$ ,  $P_{nose} = 1.27 \times 10^{-3}$ , Supplementary Table 9; Fig. 4b).

286 Next, the mean PBS values for the nose-associated loci were significantly  
287 higher than random variants in EUR ( $P = 6.90 \times 10^{-4}$ ) but not in EAS ( $P = 0.15$ )  
288 (Supplementary Table 9, Fig. 4c), indicating that nose shape may be under  
289 subtle local selection in Europeans rather than in East Asians. In addition, we  
290 found that mean PBS values for the nose-associated loci in a recent published  
291 EUR facial GWAS were significantly higher in EUR ( $P_{EUR} = 9.46 \times 10^{-3}$ ) but not  
292 in EAS ( $P_{EAS} = 0.464$ ; Fig. 4d)<sup>18</sup>. The results support that nose shape may be  
293 under local selection in EUR rather than in EAS populations.

294 Based on a study of He et al., we estimated and tested differences using the  
295 selection coefficients for nose variants with positive contribution to East Asian  
296 nose appearance (EAS-FA in the nose segment)<sup>52</sup>. The EUR population  
297 showed higher selection coefficients for nose EAS-FA increasing variants than  
298 the EAS population ( $P = 4.88 \times 10^{-2}$ , Supplementary Table 9, Fig. 4e). Moreover,  
299 by comparing the mean genetic prediction of EAS and EUR's facial variation to



300 the expected difference under random drift (Methods), the nose and glabella  
301 morphology in EUR was more protruding than EAS and the divergence of the  
302 nose was greater than expected under the neutral model (Fig. 4f)<sup>34</sup>.  
303 Furthermore, by comparing the PPS using the leading variants with the  
304 expected PPS under random drift in the EUR and EAS, we obtained the  
305 direction (and significance) of natural selection on facial morphology in each  
306 population<sup>34</sup>. Similar to the population differences, the nose, glabella, and  
307 zygoma were under significant natural selection in EUR (Fig. 4g). However, in  
308 EAS, the effects of natural selection were not significant (Fig. 4h). These results  
309 suggest that facial morphology in EUR may undergo local adaptation,  
310 producing a more protruded nose, glabella, and flatter zygoma.

311 Based on the above results, we speculate that facial features underlying EUR-  
312 EAS differences are potentially due to the adaptive selection that occurred in  
313 the EUR population, which makes European-ancestry populations have  
314 protruded and narrow noses, significantly different from those of East Asian-  
315 ancestry populations.

316

## 317 **Discussion**

318 As a large-scale East Asian population facial GWAS using a data-driven global-  
319 to-local phenotyping, our study broadens the knowledge of craniofacial  
320 genetics outside frequently investigated European-ancestry populations.  
321 Compared to previous facial GWASs, we identified 130 (out of 244) novel  
322 variants associated with typical range facial variation, which have similar  
323 biological functions as the variants identified previously<sup>1-18</sup>. A considerable  
324 number of shared variants were independently identified in a EUR study and

325 our EAS study, using the same facial phenotyping approach. Among the 114  
326 known variants, 96 were associated with consistent facial regions reported in  
327 the previous facial GWASs. When we compared shared variants with EUR  
328 study of White et al., 82 out of 89 were associated with the same facial regions,  
329 which indicates that different segmentation patterns could obtain similar GWAS  
330 results (Supplementary Table 3, 6)<sup>18</sup>. These results suggest that the 244  
331 variants identified in our study are reliable and the genetic factors underlying  
332 facial variation might be universal across populations.

333 We further extended the concepts of polygenic scores (PGS) to polygenic  
334 shapes (PS) in order to verify whether the association found in EAS could be  
335 generalized to EUR<sup>37</sup>. Both visual and statistical evidence supported this  
336 hypothesis on the whole face and major anatomical facial regions. However,  
337 the PPS of the mandible, forehead, and lower mouth showed some difference  
338 from the corresponding EUR and EAS average shapes, which is mainly due to  
339 the insufficient number of significant variants associated with these facial  
340 regions. Besides smaller phenotypic variation in these regions in EAS,  
341 environmental factors contributing to facial variation may also impact the results.  
342 In addition, the  $Q_{ST}$  analysis suggests that mandible, lower mouth, and  
343 forehead exhibit fewer signals of facial shape differences between EAS and  
344 EUR<sup>19</sup>. This could explain some reason for the inconsistency of the PPS for  
345 these facial regions. Of future interest is to calculate the PPS by combining all  
346 the variants identified in EAS and EUR studies. This might further improve the  
347 PPS in representing population facial shapes.

348 Our study also provides insights into the genetic basis of the facial shape  
349 differences between European-ancestry populations and East Asian-ancestry

350 populations. In addition to identifying 13 primary variants contributing to EUR-  
351 EAS facial differences, we provided a method to investigate the genetic factors  
352 associated with inter-population facial variation. These 13 variants all had  
353 positive and larger effects on EUR-EAS facial differences, shaping the faces of  
354 East Asian populations to be more EAS-FA. Again, corresponding with the PPS  
355 results, due to the innate limitation of GWAS, our study may overlook rare or  
356 fixed variants that lead to more EAS-FA in EAS population. By applying our  
357 method to EUR populations, additional variants affecting EAS-FA might be  
358 discovered. Moreover, for those rare or fixed variants with opposite alleles  
359 between EUR and EAS, a single population GWAS has limitations, and an  
360 admixture population instead is needed.

361 Due to the large number of significant variants identified in the nose region, our  
362 evolutionary analysis further supports the hypothesis proposed by Zaidi et al.  
363 that human nose shape has evolved in response to selection pressures<sup>53</sup>.  
364 Again, the PBS analyses showed that nose shape difference between EUR and  
365 EAS is mainly due to natural selection in European-ancestry populations rather  
366 than in East Asian-ancestry populations<sup>18</sup>.

367 In conclusion, this study presents a large EAS population GWAS on 3D facial  
368 shapes. Our study identified a large number of novel variants associated with  
369 normal range facial shape variation. Using newly introduced polygenic shapes,  
370 we successfully depicted perceptually recognizable population average faces,  
371 making our results more tangible, comprehensive, and intuitive. We identified  
372 13 variants contributing to more EAS-FA and revealed natural selection in  
373 shaping EUR-EAS nose shape difference. Our findings will greatly facilitate the  
374 understanding of human facial morphology across populations.

375

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395

396 **Author Contributions Statement**

397 S.Wang., L.J., J.L., and M.Z. conceptualized the study (ideas; formulation or  
398 evolution of overarching research goals and aims). M.Z, S.Wu., S.D., W.Q. and  
399 J.L. carried out the data curation (management activities to annotate, scrub

400 data and maintain research data for initial use and later re-use). M.Z, S.Wu.,  
401 S.D., W.Q., J.L. and J.C. carried out the formal analysis (application of  
402 statistical, mathematical, computational, or other formal techniques to analyze  
403 or synthesize study data). M.Z, S.Wu., S.D., W.Q. and J.L. did the visualization  
404 (preparation, creation and/or presentation of the published work, specifically  
405 visualization/data presentation). K.T. and L.Q., Y.Y. and J.T. collected the 3D  
406 facial scans of the TZL cohort. J.W., Z.Y., J.T, K.T. and L.Q. collected the 3D  
407 facial scans of the NSPT cohort. S.Wu, Y.L, and Q.P. contributed to generating  
408 the SNP array data. S.Wu., S.D., and J.L. registered the 3D facial scans of the  
409 Northern Han Chinese cohort and conducted the PCA of discovery cohort. N.N.  
410 and A.R.L. performed the analysis of *MPRS22/Mprs22* and human/mouse  
411 craniofacial shape. M.Z, S.Wu., S.D., W.Q. and J.L. wrote the original draft.  
412 S.Wang., L.J., P.C., J.L., M.Z, S.Wu., S.D., and W.Q. reviewed and edited the  
413 final manuscript. All authors participated in preparing the manuscript by reading  
414 and commenting on drafts before submission.

415

#### 416 **Competing Interests Statement**

417 The authors declare no competing interests.

418

419

## 420 Tables

421 Table 1: The 13 variants mainly associated with East Asian facial appearance (EAS-FA)

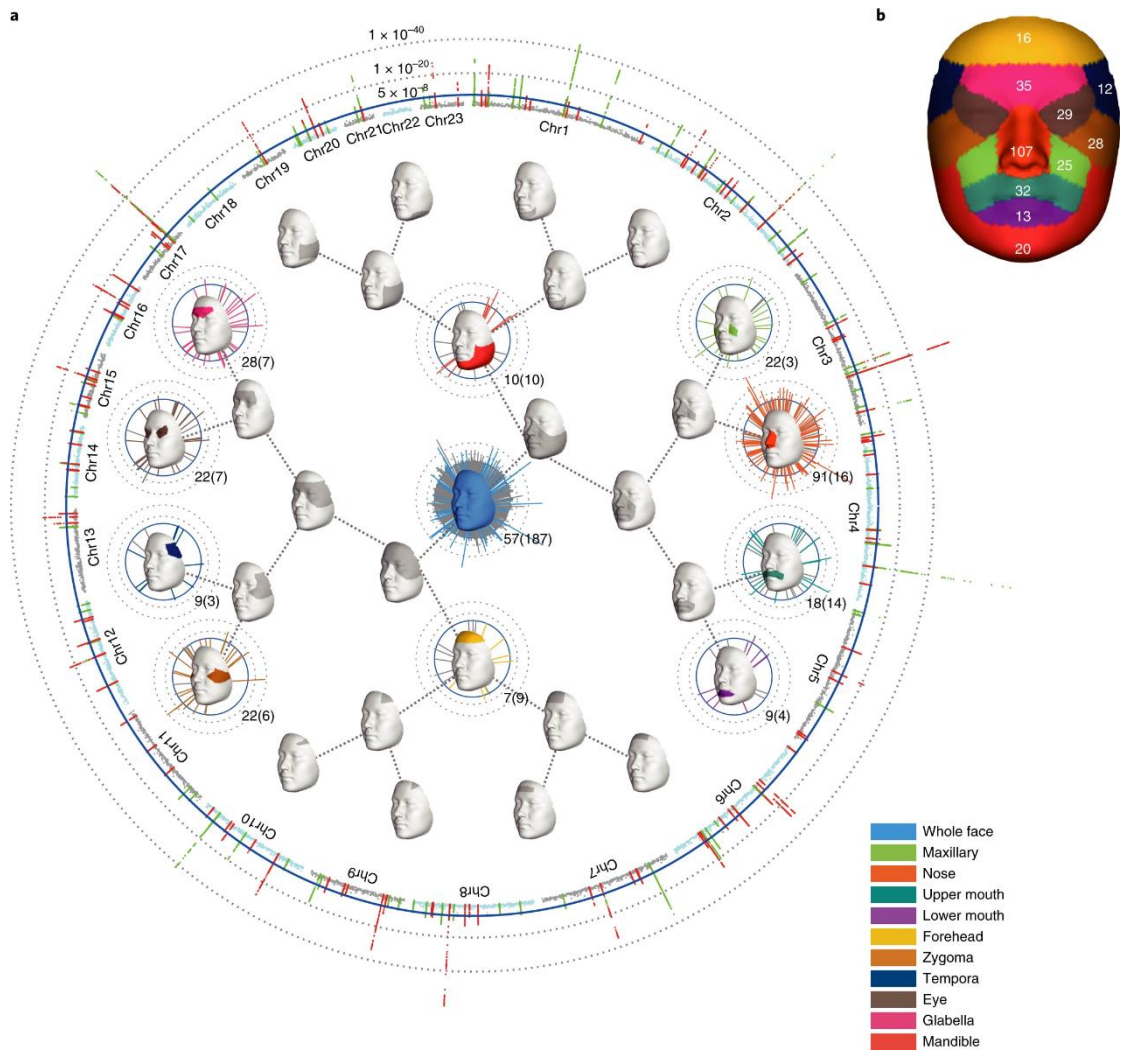
rsID	CytoBand	A1	A2	P-value	EUR	EAS	PBS_EAS	PBS_EUR	$F_{ST\_EURvEAS}$	Seg	Candidate gene	Allele age
rs7516137	1p36.32	C	G	$9.75 \times 10^{-29}$	0.318	0.553	0.077	0.036	0.107	18	<i>PRDM16</i>	537708
rs6669519*†	1p31.1	T	A	$3.40 \times 10^{-08}$	0.173	0.781	0.831	0.000	0.547	24	<i>LHX8</i>	51373
rs10923710	1p12	G	T	$1.20 \times 10^{-44}$	0.193	0.507	0.262	0.000	0.207	19, 26	<i>TBX15</i>	88185
rs3827760	2q12.3	A	G	$2.17 \times 10^{-13}$	0.000	0.921	2.587	0.306	0.945	27	<i>EDAR</i>	36410
rs12473319*¶†	2q37.1	G	C	$1.35 \times 10^{-10}$	0.022	0.478	0.554	0.161	0.511	24	<i>DIS3L2</i>	39385
rs12632544	3q23	T	A	$1.87 \times 10^{-65}$	0.000	0.500	0.571	0.332	0.595	24, 25, 26	<i>MRPS22</i>	626678
rs147468294	6q14.3	A	AC	$9.02 \times 10^{-17}$	1.000	0.690	0.299	0.207	0.397	7	<i>TBX18</i>	46640
rs111847181	8p23.1	G	GAC	$5.28 \times 10^{-09}$	0.454	0.964	0.779	0.000	0.424	18	<i>PPP1R3B</i>	788343
rs4749259*¶†	10p12.1	T	C	$3.88 \times 10^{-29}$	0.936	0.584	0.370	0.037	0.334	26	<i>MKX</i>	298528
rs12258832*¶†	10p12.1	A	G	$1.61 \times 10^{-24}$	0.892	0.690	0.133	0.005	0.129	26	<i>MKX</i>	64715
rs3740550*	10q26.11	A	G	$6.70 \times 10^{-43}$	0.994	0.875	0.127	0.029	0.145	19	<i>RAB11FIP2,</i> <i>EMX2</i>	64603
rs8068343*	17q24.3	C	T	$3.32 \times 10^{-51}$	0.959	0.462	0.470	0.281	0.528	18	<i>SOX9</i>	1261665
rs9980535*¶†	21q21.3	A	G	$3.99 \times 10^{-11}$	0.176	0.762	0.357	0.380	0.521	18	<i>LINC00161</i>	1030805

422 \* Novel variants in our GWAS finding, which are not in LD ( $r^2 < 0.1$ ) with variants reported in previous facial GWAS studies, see in ST1, ST3.

423 ¶ Novel loci in our GWAS finding, which are not within the same loci (&lt;500kb) in previous facial GWAS studies, see in ST1, ST3.

424 † Novel genes in our GWAS finding, which are not reported in previous facial GWAS studies.

425 **Figure Legends**



426

427 **Fig. 1: Overall results of genome-wide association meta-analyses in Han**

428 **Chinese cohort.** a) The Manhattan plot on the ring represents the meta-analyses P-

429 values, with chromosomes colored and labelled. P-values are  $-\log_{10}$  scaled. Using

430 500kb windows, peak variants are colored in red (novel variants) and green (known

431 variants). Inside the Manhattan plot, a binary tree of facial segments illustrates the

432 hierarchical facial segmentation up to level four. On the whole face and ten selected

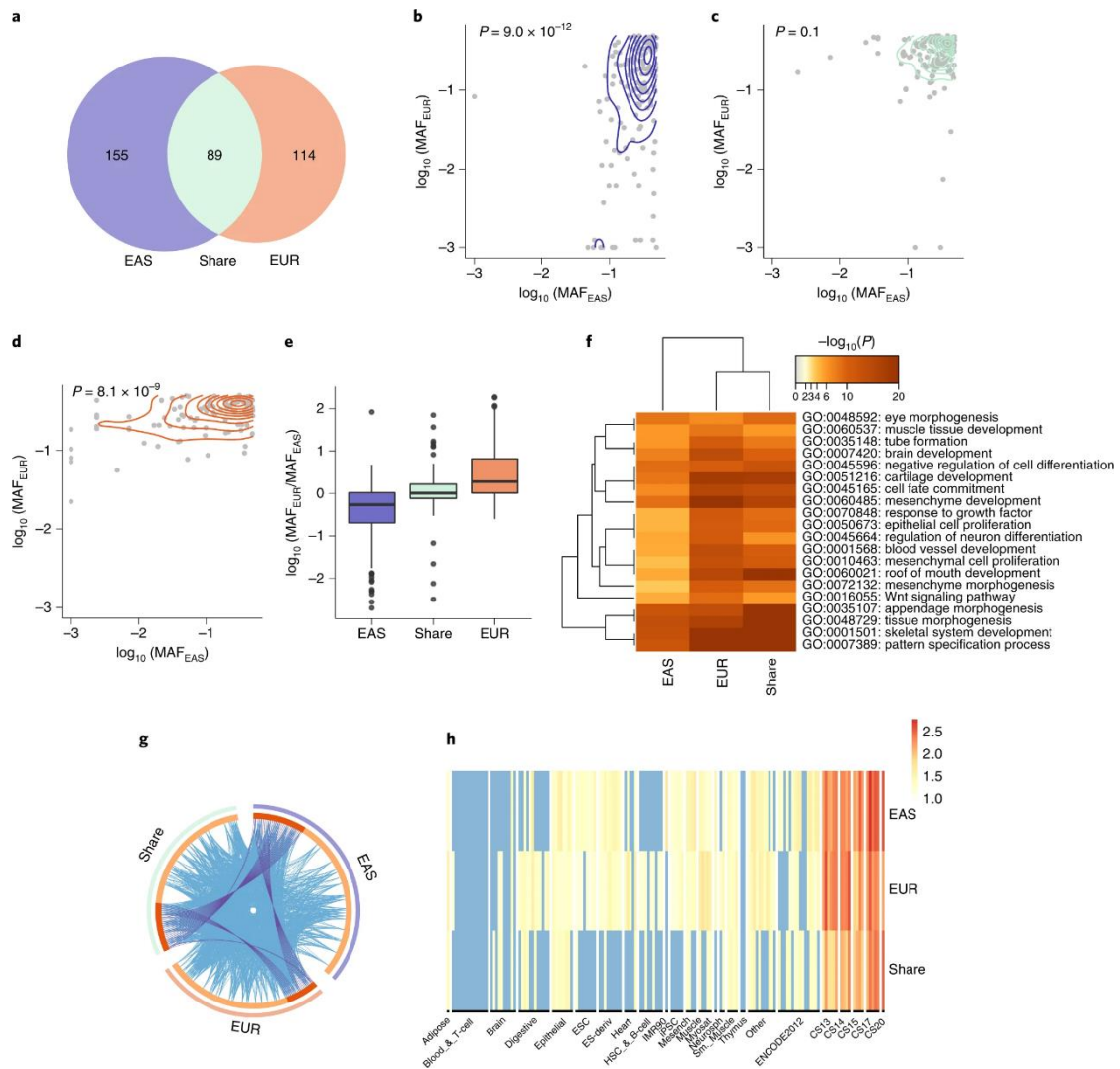
433 facial segments (colored), variants in the segment itself (colored) and in its derivative

434 segments (gray) are plotted, with the number of variants listed (#variants in derivative

435 segments in parenthesis). b) Visualization of ten selected facial segments and number

436 of significant variants.

437

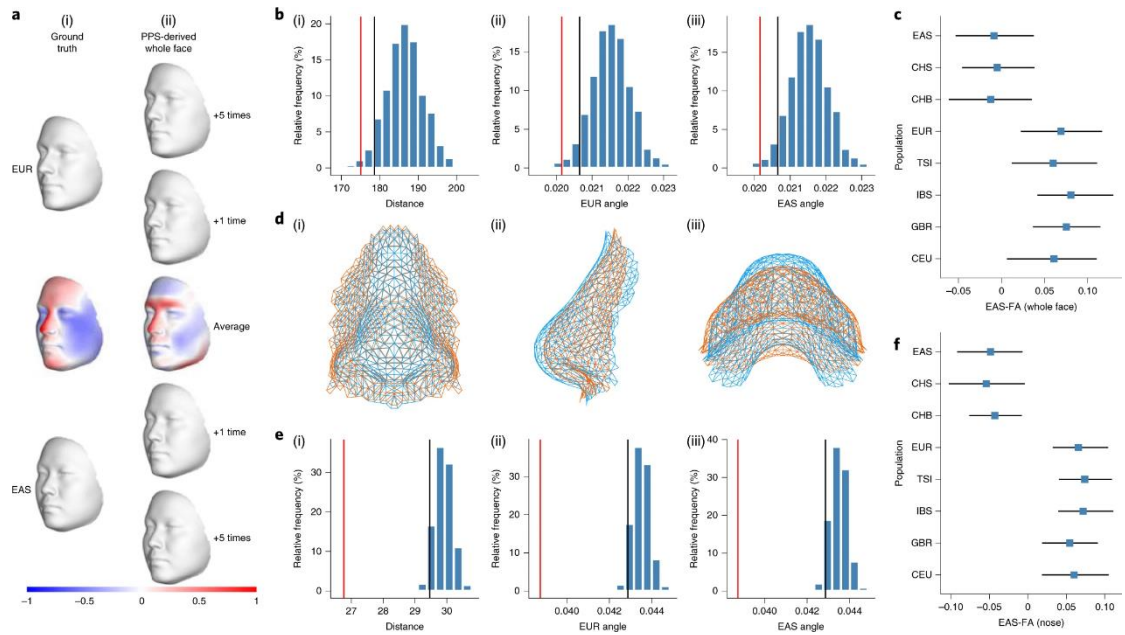


438

439 **Fig. 2: Comparison of shared and population-specific variants.** a) Number of  
 440 specific and shared variants of two study cohorts (EAS and EUR). b-e) Cross-  
 441 population MAF comparisons of b) specific variants of EAS ( $n = 155$ ). c) Shared  
 442 variants ( $n = 178$  (89 in EAS study and 89 in EUR study), shared variants in both  
 443 studies are used), and d) specific variants of EUR ( $n = 114$ ). In b-d), P-values are  
 444 provided using a two-sided Mann-Whitney U-test. When  $MAF < 0.001$ , MAF was  
 445 truncated to 0.001 to fit the log scale (1000GP). e) MAF ratio comparison of three  
 446 groups. Boxplots show medians (center lines), first and third quartiles (lower and upper  
 447 box limits, respectively), 1.5-fold interquartile ranges (whisker extents) and outliers  
 448 (black circles). Colors are corresponding with a). f) Metascape analysis shows the  
 449 biological processes associated with genes in the three groups of genes. g) Each



450 outside arc represents a group, and each inside arc represent a gene list. On the inside,  
451 each arc represents a gene list, where each gene has a spot on the arc. The dark  
452 orange color represents overlapping genes among groups. Same genes (purple lines)  
453 and different genes fell into the same ontology term (blue lines) of three groups. h)  
454 Heatmap indicates the global enrichment of trait-associated variants of each group (y  
455 axis) in enhancer of different tissue (x axis).  
456



457

458 **Fig. 3: Visualization of PPS derived whole faces and nose of EAS and EUR and**

459 **the statistical validations for PPS approach.** a) Visualization of facial morphology

460 of EAS and EUR. i) from top to bottom, representing the ground truth of EUR average

461 face, overall average face (EUR and EAS), and EAS average face respectively; ii) from

462 top to bottom, the PPS derived whole faces of 244 leading variants by adding

463  $PPS_{EUR} - PPS_{EAS}$  +5, +1 to -1, -5 times on the overall average facial shape.

464 Differences are visualized using the normal displacement (displacement in the

465 direction locally perpendicular to the facial surface), blue and red refer to depression

466 and protrusion in local shape respectively. b) The null distribution (blue) of i) Euclidean

467 distance, ii) Cosine similarity with EUR average face, and iii) Cosine similarity with EAS

468 average face using 1,000 simulations from 244 random variants on the whole face, red

469 line infers the statistics of the 244 leading variants, black line infers 95% quantile of

470 distribution from the random variants. c) The EAS-FA of polygenic shapes (whole face)

471 for individuals in 1000GP. The squares represent the mean EAS-FA score and the

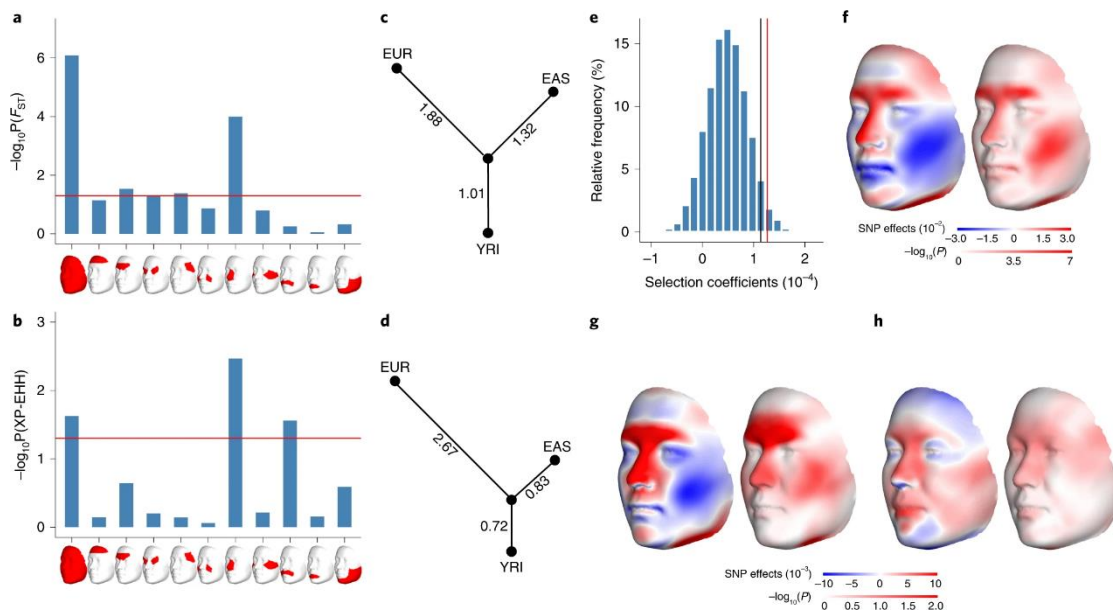
472 horizontal lines represent 1<sup>st</sup> and 3<sup>rd</sup> quantile. d) The  $PPS_{EUR} - PPS_{EAS}$  difference of

473 nose region in three views. i), ii), and iii) Front, side, and vertical views, respectively.

474 The PPS derived nose of EUR and EAS are presented in the blue and orange

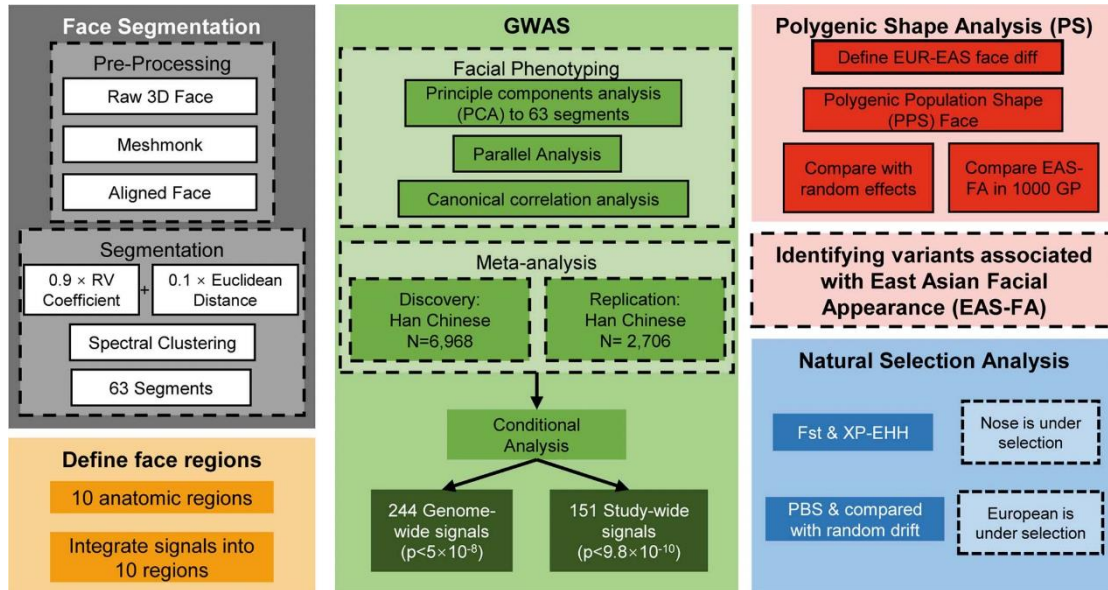
475 respectively. e) The distribution of i) Euclidean distance. ii) Cosine similarity with EUR

476 average nose and iii) Cosine similarity with EAS average nose using 1,000 simulations  
 477 from random variants, red line infers the statistics of the 107 leading variants  
 478 associated with nose, black line infers 95% quantile of distribution from the random  
 479 variants. f) The EAS-FA of polygenic shapes (nose only) for individuals in 1000GP.  
 480 The squares represent the mean EAS-FA score for the nose and the horizontal lines  
 481 represent 1<sup>st</sup> and 3<sup>rd</sup> quantile.



482

483 **Fig. 4: Natural selection analyses and enrichment test of the differentiation of**  
 484 **facial-associated variants among the EAS and EUR populations.** a, b) P-values ( $-\log_{10}$   
 485 scale) of a)  $F_{ST}$  and b) XP-EHH for the whole face and 10 anatomical regions.  
 486 The red line is the P-value threshold of 0.05. c, d) Observed mean PBS value for the  
 487 leading variants c) the 244 variants in this EAS study, and d) the 203 variants from  
 488 study of White et al against the null distribution among EAS, EUR and YRI for the nose  
 489 region. e) Selection coefficients for the nose region against the underlying null  
 490 distribution (blue). The red line corresponds to the observed selection coefficients. The  
 491 black line is 95% quantile of the null distribution. f) Differentiated accumulated genetic  
 492 effects of the 244 leading variants (visualized using the local surface normal  
 493 displacement) and P-values ( $-\log_{10}$  scale) of each quasi-landmark. g) Effects and P-  
 494 values ( $-\log_{10}$  scale) of each quasi-landmark compared with random drift in the  
 495 European population and in h) the East Asian population.



497

498

**Extended Data Fig. 1: Study design.** We first start with a face segmentation

499

procedure to get 63 face segments from which we defined 10 anatomical face regions.

500

Then by using a CCA based GWAS, we identified 244 variants with a P value lower

501

than  $5 \times 10^{-8}$ , in which 151 are also lower than  $9.8 \times 10^{-10}$ . To investigate what affects

502

the similarity of an EAS face, we used polygenic population shape (PPS) analyses to

503

fit EUR and EAS faces and identified 13 variants mainly contributing to EUR-EAS facial

504

differences. To investigate selection on facial variation, we used FST and XP-EHH to

505

find which parts of the face are under selection. These results, we further compared

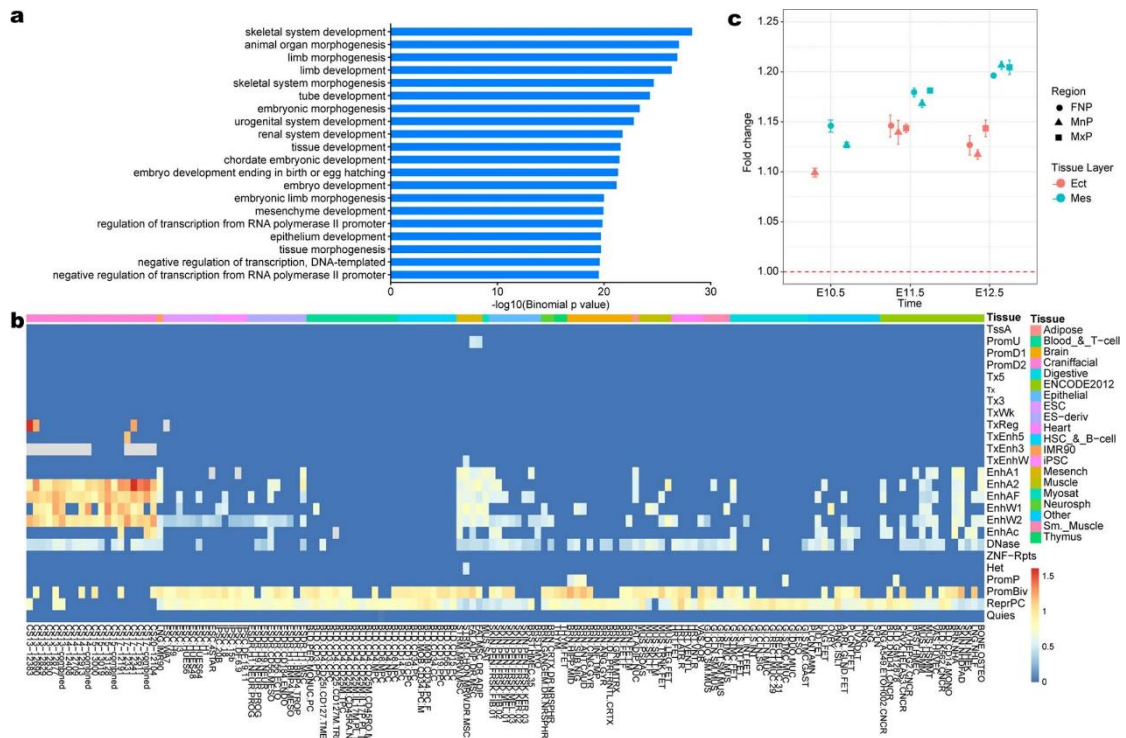
506

with random drift and random PPS to find out, which from the two populations, EUR or

507

EAS, experienced selection.

508



509  
510

**Extended Data Fig. 2: Enrichment analysis of leading variants.** (a) Geno Ontology

511 enrichment for genes annotated from leading variants by GREAT24. (b) Heatmap

512 indicating the global enrichment of trait-associated variants in different chromatin state

513 (y axis) and in different tissue (x axis). The fold change was calculated by GREGOR28.

514 The embryonic craniofacial tissue was previously published by epigenomic atlas, while

515 the other was previously published by Roadmap Epigenome<sup>27</sup>. The description of the

516 25-state chromatin model can be found at:

517 [https://egg2.wustl.edu/roadmap/web\\_portal/imputed.html#chr\\_imp](https://egg2.wustl.edu/roadmap/web_portal/imputed.html#chr_imp). (c) Expression

518 levels of the candidate genes in craniofacial tissues. Each point (n=3 biologically

519 independent replicates for each condition) represents an estimated fold change

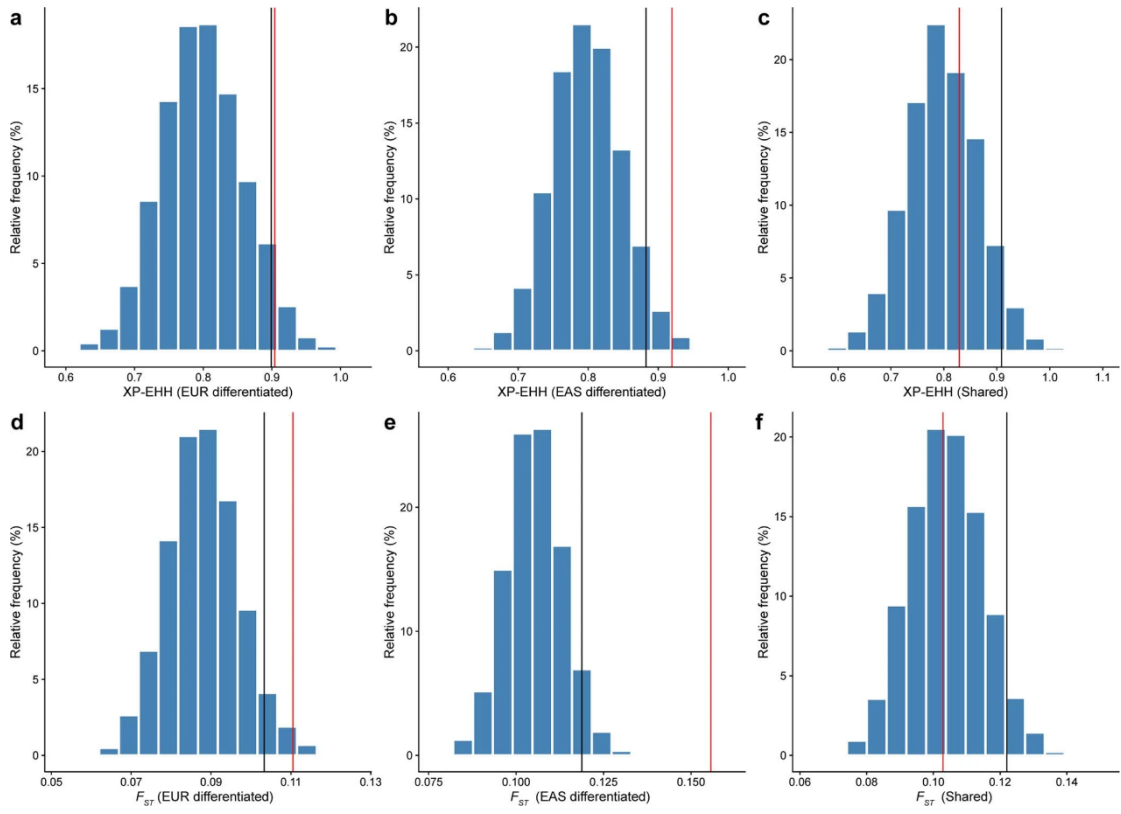
520 compared to control genes at different times (E10.5, E11.5, E12.5), in different

521 prominences (Frontonasal, FNP: circle; Maxillary, MxP: square; Mandibular, MnP:

522 triangle), and tissue layer (Ectoderm, Ect: red; Mesenchyme, Mes: blue). Data are

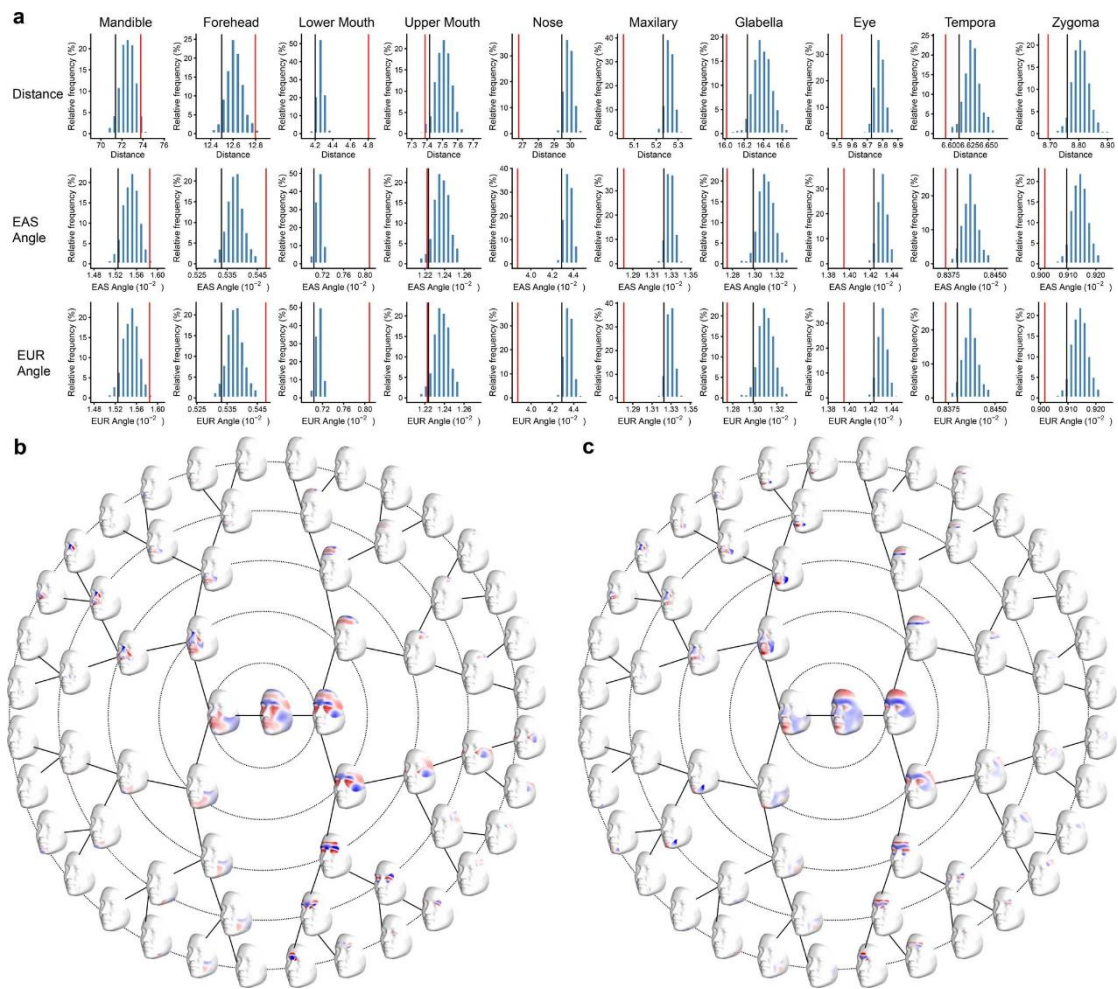
523 presented as mean values +/- 95% confidence intervals (1.96×SEM).

524



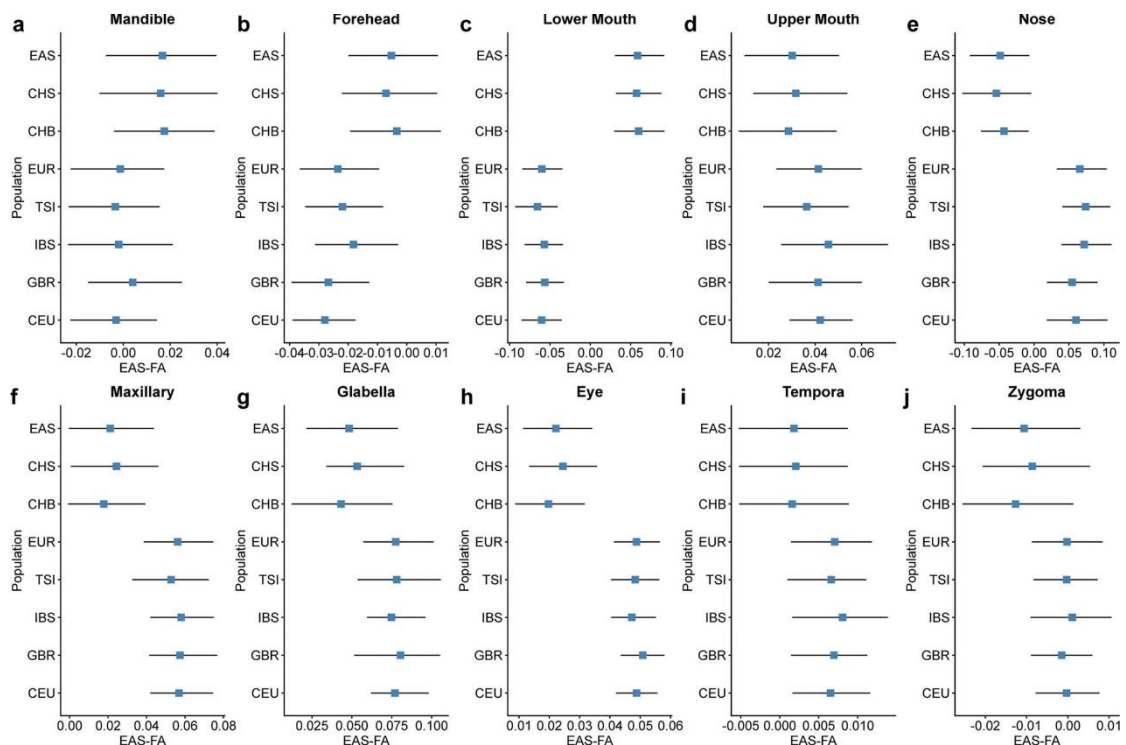


526 **Extended Data Fig. 3 XP-EHH and FST enrichment analysis for shared and**  
 527 **differentiated variants.** XP-EHH and FST enrichment analysis for (a, d) EUR  
 528 differentiated variants, (b, e) EAS differentiated variants, and (c, f) shared variants in  
 529 EAS study. The blue color is the null distribution. The red line is the mean XP-EHH or  
 530 FST score of shared or differentiated variants. The black line is the 95% quantile of the  
 531 null distribution.



532

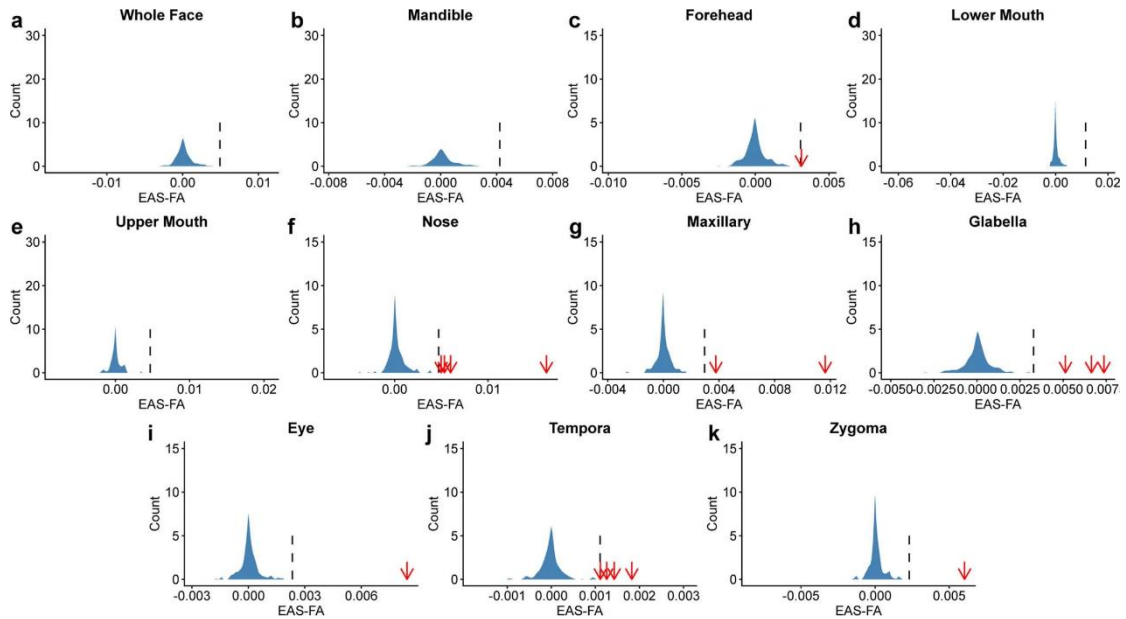
533 **Extended Data Fig. 4 Validation of PPS in 10 anatomical segments.** (a) The null  
 534 distribution (blue) of Euclidean distance, cosine similarity with EUR average face and  
 535 EAS average face using 1,000 simulations derived from random variants on the 10  
 536 anatomical regions, red line infers the statistics of the leading variants associated with  
 537 corresponding regions; black line infers 95% quantile of distribution from the random  
 538 variants with corresponding regions; (b) The genetic effects of rs12632544 and (c)  
 539 rs12473319 weighted by their effect allele number difference of EUR and EAS  
 540 (visualized using the local surface normal displacement).



541 **Extended Data Fig. 5 The EAS-FA of polygenic shapes in 10 anatomical regions**  
 542 **for EAS and EUR individuals in 1000GP.** The EAS-FA of polygenic shapes in a)  
 543 mandible, b) forehead, c) lower mouth, d) upper mouth, e) nose, f) maxillary, g) glabella,  
 544 h) eye, i) tempora, and j) zygoma for EAS and EUR individuals in 1000GP. The squares  
 545 represent the mean EAS-FA score in 10 anatomical regions and the horizontal lines  
 546 represent 1st and 3rd quantile.  
 547

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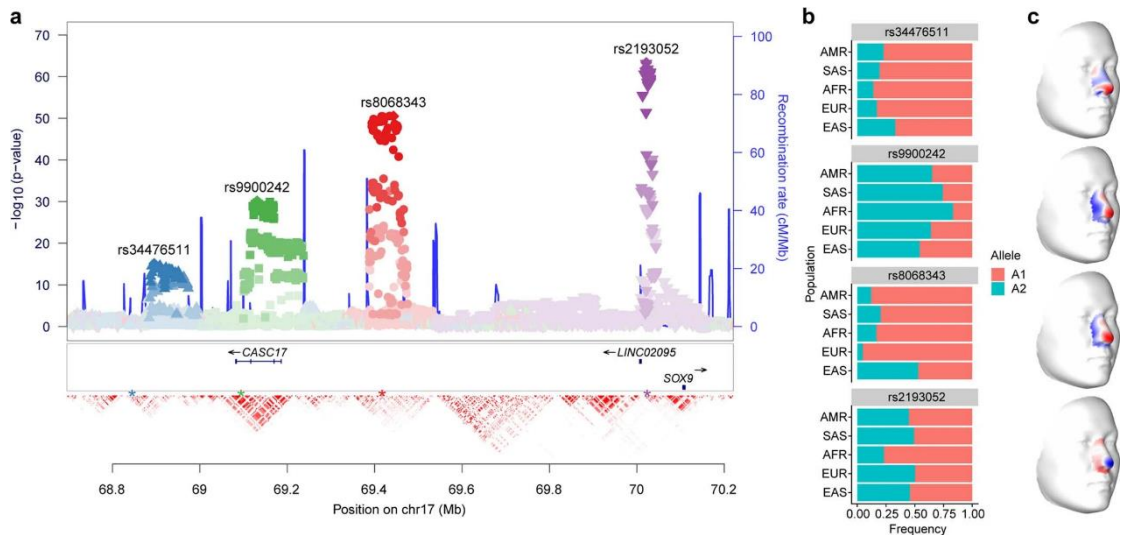


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**Extended Data Fig. 6 EAS-FA of the 244 leading variants on the EUR-EAS**

551 **difference.** The distributions (blue) of EAS-FA derived from 244 leading variants  
552 associated with a) whole face and b) - k) 10 anatomical segments. The black dotted  
553 line is the EAS-FA threshold of each region (mean + 3×SD). The red arrow is the variant  
554 over threshold.

555



556

557 **Extended Data Fig. 7 Multi peak in 17q24.3 region.** (a) Association variants in the  
558 SOX9 locus and genomic environment surrounding SOX9 across a 2-Mb window. Four  
559 independent variants, represented by (1) rs34476511 (blue), (2) rs9900242 (green), (3)  
560 rs8068343 (red), and (4) rs2193052 (purple) are observed; (b) Allele frequency in AMR,  
561 SAS, AFR, EUR and EAS population of the four variants from 1000GP; (c) The effects  
562 of the four variants in the nose region.

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564

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- 695

696 **Materials and methods**

697 **Ethics statement**

698 All participants provided written informed consent, and all study protocols were  
699 approved by the institutional review boards of the pertinent research institutions.  
700 The National Survey of Physical Traits (NSPT) is the sub project of The National  
701 Science & Technology Basic Research Project which was approved by the  
702 Ethics Committee of Human Genetic Resources of School of Life Sciences,  
703 Fudan University, Shanghai (14117). The Northern Han Chinese (NHC) cohort  
704 was approved by the Ethics Committee of Human Genetic Resources at the  
705 Shanghai Institute of Life Sciences, Chinese Academy of Sciences (ER-SIBS-  
706 261410-A1801). The Taizhou Longitudinal Study (TZL) was approved by the  
707 Ethics Committee of Human Genetic Resources at the Shanghai institute of life  
708 Sciences, Chinese Academy of Sciences (ER-SIBS-261410). Written informed  
709 consent was granted for each participant before enrollment in the study. We  
710 confirm that our study is compliant with the Guidance of the Ministry of Science  
711 and Technology (MOST) for the Review and Approval of Human Genetic  
712 Resources.

713

714 **Sample and recruitment details**

715 The samples in this study were collected from three independent cohorts, the  
716 National Survey of Physical Traits (NSPT) cohort ( $n = 3,322$ ), the Northern Han  
717 Chinese (NHC) cohort ( $n = 4,767$ ), and the Taizhou Longitudinal Study (TZL)  
718 cohort ( $n = 2,881$ ). For the NSPT sample, individuals were recruited at three  
719 Chinese cities: Nanning, Guangxi province ( $n = 1,326$ ); Taizhou, Jiangsu  
720 Province ( $n = 986$ ); Zhengzhou, Henan province ( $n = 1,010$ ). In the NHC cohort,

721 participants were recruited in Tangshan, Hebei province. These two cohorts  
722 constituted the discovery dataset. The TZL cohort, where individuals were  
723 recruited in Taizhou, Jiangsu province, were used as the replication dataset.  
724 The characteristics of the datasets is shown in Supplementary Table 2.

725

### 726 **Genotyping and imputation**

727 Since we used two different genotyping platforms in the discovery and  
728 replication datasets (details in the Supplementary Note), we chose to impute  
729 the two data sets separately, then combine the imputed results.

730 For each dataset, standard data cleaning and quality assurance practices were  
731 performed based on the GRCh37 genome assembly. Phasing was performed  
732 using SHAPEIT2 (v2.17)<sup>54</sup> and imputation to the 1000GP Phase 3 reference  
733 panel using IMPUTE2 (v2.3.2)<sup>55</sup>. After post-imputation quality control,  
734 8,018,212 shared variants were obtained for analysis.

735

### 736 **3D image acquisition, registration, and quality control**

737 3D images of all individuals in the three cohorts were captured and acquired  
738 using the 3dMDface (3dMD) camera system. When capturing, participants were  
739 asked to close mouth, open eyes, and hold faces with a neutral expression.

740 The 3D surface images were registered using the MeshMonk (v0.0.6)<sup>56</sup> in  
741 MATLAB™ 2018a. This performed a homologous configuration of 7,906  
742 spatially dense landmarks, allowing the 3D image data to be standardized. We  
743 performed Generalized Procrustes analysis (GPA) and symmetrization, then  
744 investigated every mapped image manually and identified outlier images. 3D

745 facial images with poor quality were removed or re-processed, with details  
746 available in the Supplementary Note.

747 As a result, 6,968 ( $n = 4,089$  in NHC cohort,  $n = 2,879$  in NSPT cohort) and  
748 2,706 unrelated individuals with good quality 3D images in the discovery and  
749 replication dataset are used for further analysis.

750

### 751 **Facial phenotyping**

752 Like the approach of White et al., we performed a semi-supervised facial  
753 segmentation based on the phenotypic correlation between facial landmarks  
754 using the discovery dataset<sup>18</sup>. To calculate the phenotypic correlations, we first  
755 corrected the symmetrized facial shapes for the covariates of age, age squared,  
756 sex, body mass index (BMI), and four SUGIBS components using a partial  
757 least-squares regression (PLSR, function `plsregress` from MATLAB™ 2018a) in  
758 both the discovery and replication cohort<sup>18</sup>. SUGIBS is a method for genetically  
759 robust genome-wide ancestry inference based on the spectral (S)  
760 decomposition of an unnormalized genomic (UG) relationship matrix  
761 generalized by an Identity-by-State (IBS) similarity degree of individuals' matrix,  
762 which was also used by White et al<sup>18,57</sup>.

763 To study global and local effects on facial variation, we refined the data-driven  
764 facial segmentation method to avoid isolated point and cluster specific facial  
765 regions. We performed a hierarchical spectral clustering on a combined matrix,  
766 as  $0.9 \times RV$  similarity matrix +  $0.1 \times$  distance matrix, up to level five, resulting in a  
767 total of 63 facial segments (Supplementary Fig. 6, Supplementary Note). In  
768 each segment, we performed principal component analysis (PCA) on the PLSR  
769 residuals of the discovery cohort and obtained the PC scores as the phenotypic

770 scores for the discovery cohort. In the replication cohort, we projected the PLSR  
771 residuals onto the PCA space build in the discovery cohort to obtain the PC  
772 scores as the phenotypic scores. We described the methods in detail in the  
773 Supplementary Note.

774

### 775 **Multivariate genome-wide association meta-analyses**

776 The association analysis is similar to that in White et al<sup>18</sup>. For all three phases  
777 (discovery, replication, and meta-analysis), the genotypes were coded as the  
778 number of major alleles present (0, 1 or 2). In the discovery phase, in each of  
779 the 63 facial segments, we used canonical correlation analysis (CCA) to define  
780 the linear combination of the facial segments PCs that are mostly correlated  
781 with each variant, which represent the phenotypic effect in shape space. When  
782 one of the two sets of variables has only one variable, CCA reduces to multiple  
783 regression<sup>58</sup>. The resulting vector  $\omega_i$  is the effect size vector of this variant in  
784 the shape PCA space (Supplementary Note). The correlation can be tested for  
785 significance based on Rao's exact F-test (one-sided, right tail)<sup>59</sup>. For each  
786 variant, we obtained a direction  $\omega_i$  in the shape PCA space most correlated  
787 with the genotype of that variant and a P-value representing the strength of  
788 correlation in the discovery phase. In the replication phase, we first projected  
789 the phenotypic scores onto the CCA direction and calculated the Pearson's  
790 correlation between the projected scores and the genotypes in the replication  
791 cohort. To test the correlation's significance, we used the Student's t-test where

792 the t-statistics is defined as  $t = \frac{\sqrt{\rho^2(1-\rho^2)}}{\sqrt{n-2}}$ . We performed a one-sided right

793 tail test for each variant to ensure that the effective direction of the variant within



794 the two datasets is the same. Next, the P-values obtained in the discovery and  
795 replication phase were combined in a meta-analysis using Stouffer's method  
796 weighted by the sample sizes<sup>22</sup>. We used the corresponding implementations  
797 of these methods in the SNPLIB package (available at [https://github.com/jiarui-](https://github.com/jiarui-li/SNPLIB)  
798 [li/SNPLIB](https://github.com/jiarui-li/SNPLIB)) to accelerate the analyses.

799

### 800 **Conditional analysis and GWAS peak selection**

801 For every variant, the meta-analysis described above yielded 63 P-values  
802 representing 63 facial segments. In the conditional analysis and peak selection,  
803 we selected the lowest P-value for each variant. For the initial selection, we  
804 selected the variants with P-value below the genome-wide threshold ( $P = 5 \times 10^{-8}$ )  
805 and calculated the pairwise  $r^2$  between these variants. In each chromosome,  
806 we grouped the selected variants consecutively in a way that the  $r^2$  between  
807 every two neighbor selected variants in the group is larger than 0.05, which  
808 resulted in 230 groups. Then, we selected the variant with the lowest P-value  
809 as the conditional variant for each group and performed association tests of the  
810 remaining variants on the condition of the conditional variant. The variant with  
811 the most significant P-value still lower than the genome-wide threshold was  
812 selected as conditional variants. We repeated these two steps until no variant  
813 remains significant. Finally, we obtained 244 leading SNPs from all groups. We  
814 considered  $\pm 500$  kb genomic region of each leading variant as a genomic locus.  
815 If nearby genomic regions overlapped, we merged them into one genomic locus.  
816

### 817 **Permutation test of study-wide P-value threshold**

818 To determine the study-wide Bonferroni P-value threshold, we calculated the  
819 number of independent tests by both the eigenvalues of the correlation matrix  
820 of the segments and the permutation analysis scheme used in the study of  
821 White et al<sup>18,20,21</sup>. The numbers of independent tests obtained from the  
822 eigenvalues of the correlation matrix and the permutation analysis are 50 and  
823 51.41, respectively. Here, we used the more stringent threshold  $5 \times 10^{-8} / 51.41 =$   
824  $9.8 \times 10^{-10}$ . The details can be found in the Supplementary Note.

825

### 826 **Heritability of Facial Segments**

827 In each facial segment, we first calculated the genome-wide heritability of each  
828 retained shape PCs using the discovery cohort and calculated the facial  
829 segment's heritability as the mean of the PCs' heritability weighted by each  
830 PC's eigenvalue (variance). To calculate the genomic relationship matrix (GRM)  
831 for heritability estimation, we first removed SNPs based on high levels of  
832 pairwise LD by PLINK v1.9 with window size of 50, step size of 5 bases, and  
833  $r^2 > 0.1$ , remaining 266,241 SNPs. The heritability of all facial segment PCs was  
834 estimated by GCTA<sup>60,61</sup>.

835

### 836 **Gene mapping and functional annotation**

837 Candidate genes of the genome-wide-significant leading SNPs were first  
838 identified using the NCBI, HaploReg V4.1, UCSC genome browser and  
839 Ensemble genome browser<sup>62-65</sup>. We also used three gene-mapping criteria  
840 implemented in Functional Mapping and Annotation (FUMA, v1.3.6) to identify  
841 the most likely candidate gene per leading variant<sup>25</sup>. First, we map variants to

842 genes based on physical distance (within a 10,000 base pair window) from the  
843 known protein-coding genes in the human reference assembly. Second, we  
844 included the genes which have a significant cis- expression quantitative trait  
845 locus (eQTL; 1 Mb distance to the leading variant) association with the leading  
846 variants, using 10 tissue types from the GTEx v8 database<sup>66-68</sup>. We used an  
847 FDR of 0.05 to define significant eQTL associations. Finally, we also identified  
848 candidate genes for each leading variant if there is chromatin interaction. To  
849 further prioritize candidate genes, we limited interaction-mapped genes to those  
850 who interact with a predicted enhancer region identified in any of the 111 tissues  
851 or cell types from the Roadmap Epigenomics Mapping Consortium (ROADMAP)  
852 and/or a gene promoter region (from 250 bp upstream to 500 bp downstream  
853 of the transcription start site (TSS) and predicted by the ROADMAP to be a  
854 promoter region)<sup>27</sup>. We expected that the resulting candidate genes are more  
855 likely to have a plausible biological function. We used an FDR of  $1 \times 10^{-6}$  to  
856 define significant interactions. To further narrow down the candidate genes, we  
857 investigated whether any gene in the window was previously associated with  
858 craniofacial development or morphology through normal-range facial  
859 association studies, genetic disorders with facial dysmorphology phenotypes,  
860 or animal models. To investigate the potential biological process of the  
861 candidate genes, FUMA (v1.3.6) and GREAT (v4.0.4) were performed using  
862 preset parameters<sup>24,25</sup>.

863

#### 864 **Chromatin state association in embryonic craniofacial tissue**

865 We used GREGOR (v1.4.0) to evaluate global enrichment of trait-associated  
866 variants in different chromatin states<sup>28</sup>. This method tests for an increase in the

867 number of facial-associated index variants, or their LD proxies ( $r^2 > 0.8$ ),  
868 overlapping with the regulatory feature more often than expected by chance by  
869 comparing to permuted control sets (random control variants are selected  
870 across the genome that match the index variant for several variants in LD, minor  
871 allele frequency and distance to nearest intron). The reference epigenomes of  
872 127 human tissues and cell types were obtained from the NIH Roadmap  
873 Epigenomics Mapping Consortium<sup>26</sup>. The human embryonic craniofacial  
874 chromHMM states were obtained from each Carnegie stage by Wilderman et  
875 al<sup>29</sup>.

876

#### 877 **Gene expression enrichment analysis**

878 We selected a set of transcriptome datasets from critical periods of mouse face  
879 formation that enable gene expression to be analyzed with respect to time,  
880 prominence, and tissue layer (GSE62214). We evaluate the expression level  
881 (fold change) of the candidate genes compare to a set of control groups where  
882 the genes were randomly selected from the genome. Then we regressed the  
883 fold change of gene expression on time, prominence, and tissue layer to test  
884 their associations.

885

#### 886 **The EAS and EUR average facial shapes**

887 We recruited 89 individuals with self-reported European ancestry (32 females  
888 and 57 males) between 16 and 57 years old in Shanghai<sup>25</sup>. They were required  
889 to have complete European ancestry over the last three generations. Their 3D  
890 facial images were captured using the same protocol as used in the Chinese  
891 cohort. In each segment, we aligned these EUR facial shapes to the

892 corresponding sample full Procrustes mean shape. We then calculated the  
 893 male and female average facial shapes separately and used the average facial  
 894 shapes of the two average shapes as the EUR average facial shapes. To  
 895 calculate the EAS average facial shapes, we selected five individuals in the Han  
 896 Chinese cohort with matched age and gender to the individuals in the EUR  
 897 cohort. We finally selected 445 (5×89) individuals to calculate the EAS average  
 898 facial shapes in the same manner as in the EUR cohort.

899

### 900 **Polygenic shape analysis (PSA)**

901 One can calculate the effect size vector  $\beta_i$  in the original shape space by:

$$902 \quad \beta_i = \mathbf{V}_k \Sigma_k \omega_i \quad (1)$$

903 where  $\Sigma_k$  is a diagonal matrix of the largest  $k$  singular values and the column  
 904 vectors of  $\mathbf{V}_k$  are the corresponding  $k$  right singular vectors obtained from the  
 905 PCA (Supplementary Note).

906 Thus, the polygenic shape (PS) of an individual could be calculated as:

$$907 \quad \mathbf{PS} = \sum_i^n \beta_i g_i \quad (2)$$

908 where  $g_i$  is the genotype value of variant  $i$ <sup>37</sup>. Subsequently, we calculate the  
 909 polygenic population shape (PPS) by:

$$910 \quad \mathbf{PPS} = 2 \sum_i^n \beta_i a_i \quad (3)$$

911 where  $a_i$  is the effect allele frequency of variant  $i$  and two times  $a_i$  is the  
 912 average number of effect alleles in a given population.

913 Next, we used the PPS difference between EUR and EAS ( $PPS_{EUR} - PPS_{EAS}$ )  
 914 calculated by leading variants compared with random variants to evaluate

915 whether leading variants could effectively fit the EUR-EAS shape difference.

916 We calculated the PPS derived shapes as following:

$$917 \quad F_{EAS}^d = F_{AVG} - \frac{PPS_{EUR} - PPS_{EAS}}{2} \quad (4)$$

$$918 \quad F_{EUR}^d = F_{AVG} + \frac{PPS_{EUR} - PPS_{EAS}}{2} \quad (5)$$

919 where  $F_{EAS}^d$  and  $F_{EUR}^d$  are the corresponding PPS derived shapes,  $F_{AVG}$  is the  
920 average facial shape of the population average shapes of EUR and EAS (i.e.,  
921 a population neutral average face).

922 We performed 1,000 simulations to calculate the random  $PPS_{EUR} - PPS_{EAS}$ . In  
923 each simulation, random variants with the same effect allele frequencies in EAS  
924 were chosen to calculate random  $PPS_{EUR} - PPS_{EAS}$ . Subsequently, the cosine  
925 similarity and the Euclidean distances between the PPS and the corresponding  
926 average face were used as measures of shape similarity. P-values of each  
927 approach were then calculated using the null distribution established by these  
928 1,000 simulations.

929

### 930 **Variant's contribution to EAS-FA**

931 We used the projected (vector) length to quantify a variant's contribution to the  
932 EUR-EAS face difference:

$$933 \quad l_i = \frac{2(a_i^{EUR} - a_i^{EAS}) \times \beta_i \times (\bar{F}_{EUR} - \bar{F}_{EAS})}{|\bar{F}_{EUR} - \bar{F}_{EAS}|} \quad (6)$$

934 If a variant has a positive sign of projected length, we regard this variant to be  
935 linked to EAS individuals having more EAS features. In contrast, a variant with  
936 a negative sign is linked with EAS individuals having more EUR characteristic  
937 features.

938

### 939 **Genetic information used from 1000 Genome Project**

940 We used the individuals' genetic information from 1000 GP Phase 3 for related  
941 analysis including calculating MAF, PPS analysis,  $F_{ST}$  and PBS calculation, etc.  
942 EAS including Han Chinese in Beijing (CHB,  $n = 103$ ), and Southern Han  
943 Chinese (CHS,  $n = 105$ ); EUR including Toscani (TSI,  $n = 107$ ), British (GBR,  
944  $n = 91$ ), Iberian (IBS,  $n = 107$ ), and Utah residents (CEU,  $n = 99$ ), without  
945 Finnish (FIN); AFR including Yoruba (YRI,  $n = 103$ ) were used for analysis.

946

### 947 **Calculation of natural selection signatures**

948 We calculated genome-wide natural selection signatures based on XP-EHH  
949 using REHH2 (v3.2.0)<sup>36</sup>. The genome-wide XP-EHH z-scores were  
950 standardized through normalization within each derived allele frequency bin  
951 (bin widths = 0.01). We estimated two-tailed P-values of the variant according  
952 to the normalized z-scores. We calculated the  $F_{ST}$  and PBS for different sets of  
953 a population, by using the observed allele frequencies estimated from the  
954 1000GP Phase 3<sup>33,38</sup>. On the basis of a previous study, we measured selective  
955 pressures by (genic) selection coefficients, which the details of the calculations  
956 are described in He et al<sup>52</sup>.

957

### 958 **Phenome-wide selection signature analysis**

959 Similar to the approach used in Guo et al., we compared the mean  $F_{ST}$ /PBS  
960 value of the leading variants with that of the control variants with MAF and LD  
961 score matched<sup>51</sup>. First, we divided all the variants (1000GP) into 20 MAF bins  
962 from 0 to 0.5 with an increment of 0.025 (excluding the SNPs with  $MAF < 0.01$ ).  
963 Each of the MAF bins was further grouped into 20 bins according to the 20

964 quantiles of LD score distribution. The MAF and LD score values were  
965 computed from the EAS or EUR samples in the 1000GP described above.  
966 Second, we allocated the leading variants to the MAF and LD stratified bins,  
967 randomly sampled a matched number of "control" variants from each bin,  
968 computed a mean  $F_{ST}/PBS$  value for the control variants sampled from all bins,  
969 and repeated this process 10,000 times to generate a distribution of mean  
970  $F_{ST}/PBS$  under drift. Third, a P-value was computed from a two-tailed test by  
971 comparing the observed mean  $F_{ST}/PBS$  value for the leading variants against  
972 the null distribution quantified by the control variants, assuming normality of the  
973 null distribution. Regarding enrichment analysis of the selection signatures by  
974 XP-EHH, we obtained the sum of the squared values of the normalized XP-  
975 EHH z-scores of the variants (or the proxy variants in LD when available;  $r^2 >$   
976 0.6 in the CHB or CEU data from 1000 Genome Project), which was compared  
977 with the  $X^2$  distribution with the degree of freedom equal to the number of the  
978 variants.

979

### 980 **Direction of genetic differentiation**

981 The analysis below uses a similar method introduced in Robinson et al. to  
982 quantify the population genetic differentiation of a complex trait<sup>34</sup>. The leading  
983 variants' coefficients were randomized across variants 10,000 times, and  
984 10,000 genetic predictors were created in the EAS or EUR samples from the  
985 1000GP described above. By keeping the effect sizes consistent but attributing  
986 these effects across variants at random, the genetic predictors generated  
987 reflect the action of genetic drift.

988



989 **Data Availability**

990 The Meta-analysis GWAS summary statistics are available on the National  
991 Omics Data Encyclopedia.

992 NODE: OEP002283 (<https://www.biosino.org/node/project/detail/OEP002283> ).

993 The participants making up the NSPT, NHC and TZL datasets were not  
994 collected with broad data sharing consent. Given the highly identifiable nature  
995 of both facial and genomic information and unresolved issues regarding risk to  
996 participants, we opted for a more conservative approach to participant  
997 recruitment. Broad data sharing of the raw data from these collections would  
998 thus be in legal and ethical violation of the informed consent obtained from the  
999 participants. This restriction is not because of any personal or commercial  
1000 interests. Additional details can be requested from L.J. for the NSPT dataset,  
1001 and S.Wang for the NHC and TZL datasets. Data usage shall be in full  
1002 compliance with the Regulations on Management of Human Genetic Resources  
1003 in China.

1004 Publicly available data used were: the 1000 GP Phase 3 data:

1005 (<https://www.internationalgenome.org/category/phase-3/>)<sup>23</sup>, The Roadmap

1006 Epigenomics Project: (<http://www.roadmapepigenomics.org>)<sup>26</sup>, NCBI dbSNP

1007 database: (<http://www.ncbi.nlm.nih.gov/SNP>)<sup>62</sup>, UCSC genome browser:

1008 (<http://genome.ucsc.edu>)<sup>63</sup>, HaploReg v4.1:

1009 (<https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php>)<sup>64</sup>, Ensemble

1010 genome browser: ([http://asia.ensembl.org/Homo\\_sapiens/Info/Index](http://asia.ensembl.org/Homo_sapiens/Info/Index))<sup>65</sup>, GTEx

1011 v8: (<https://gtexportal.org/home/>)<sup>66-68</sup>, Human genome dating:

1012 (<https://human.genome.dating/>)<sup>49</sup>, and the transcriptome resource from

1013 separated ectoderm and mesenchyme of the developing mouse face

1014 (GSE62214):

1015 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62214>).

1016

1017 **Code availability**

1018 The statistical analyses in this work were based on functions of the statistical

1019 toolbox in MeshMonk (<https://github.com/TheWebMonks/meshmonk>,

1020 v0.0.6)<sup>56</sup>, MATLAB™ 2018a, R (v3.6.1), ggplot2 (v3.1.0), Python (v3.5.0),

1021 PLINK v1.9, SHAPEIT2 (v2.17), IMPUTE2 (v2.3.2), SNPLIB

1022 (<https://github.com/jiarui-li/SNPLIB>), GCTA-GREML, FUMA (v1.3.6), GREAT

1023 (v4.0.4), GREGOR (v1.4.0), Metascape (<https://metascape.org>), LocusZoom

1024 (<https://genome.sph.umich.edu/wiki/LocusZoom>), and REHH2 (v3.2.0) as

1025 mentioned throughout the Methods.

1026

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