

# 1 **Detecting genotype-population interaction effects by ancestry principal** 2 **components**

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26 **ABSTRACT**

27 Heterogeneity in the phenotypic mean and variance across populations is often observed for  
28 complex traits. One way to understand heterogeneous phenotypes lies in uncovering  
29 heterogeneity in genetic effects. Previous studies on genetic heterogeneity across populations  
30 were typically based on discrete groups of population stratified by different countries or  
31 cohorts, which ignored the difference of population characteristics for the individuals within  
32 each group and resulted in loss of information. Here we introduce a novel concept of  
33 genotype-by-population (G×P) interaction where population is defined by the first and second  
34 ancestry principal components (PCs), which are less likely to be confounded with  
35 country/cohort-specific factors. We applied a reaction norm model fitting each of 70 complex  
36 traits with significant SNP-heritability and the PCs as covariates to examine G×P interactions  
37 across diverse populations including white British and other white Europeans from the UK  
38 Biobank ( $N = 22,229$ ). Our results demonstrated a significant population genetic  
39 heterogeneity for behavioural traits such as age first had sexual intercourse and qualifications.  
40 Our approach may shed light on the latent genetic architecture of complex traits that underlies  
41 the modulation of genetic effects across different populations.

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## 51 **Introduction**

52 Most human traits are polygenic and their phenotypes are typically influenced by numerous  
53 genes and environmental factors, and possibly by their interactions, e.g. genotype-  
54 environment (G×E) interaction<sup>1-4</sup>. These traits have been termed as “complex traits”, which  
55 are distinguished from Mendelian traits that are shaped by a single or few major genes<sup>5</sup>.  
56 Genome-wide association studies (GWAS) have successfully discovered thousands of  
57 associations between single-nucleotide polymorphisms (SNPs) and complex traits, which  
58 have revolutionized our understanding of the polygenic architecture of complex traits<sup>6-8</sup>.  
59 Subsequently, in order to increase the power and precision to identify more causal variants,  
60 there have been numerous follow-up studies using meta-analyses of GWAS summary  
61 statistics or mega-analyses of multiple GWAS by combining diverse data sources that usually  
62 span across different nations or populations<sup>9,10</sup>. However, many human complex traits (e.g.,  
63 height and body mass index (BMI)) are substantially differentiated among diverse  
64 populations<sup>11</sup>. For instance, the mean height across European nations generally increases  
65 with latitude<sup>12</sup>. Although across-population differences in the mean values are often  
66 observed for the phenotypes of complex traits, the underlying genetic and environmental  
67 bases remain largely unknown<sup>12</sup>.

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69 One way to understand such phenotypic heterogeneity lies in uncovering genetic  
70 differentiation for the traits captured by common variants across populations. Some studies<sup>12-</sup>  
71 <sup>15</sup> have focused on examining population genetic differentiation for several anthropometric,  
72 behavioural and psychiatric phenotypes, using whole-genome statistical methods such as  
73 applying bivariate genomic restricted maximum likelihood (GREML) to estimate genetic  
74 correlation between samples from the USA and Europe for height and BMI<sup>14</sup> or determining  
75 interaction of genotype by seven sampling populations for behavioural traits by a G-C

76 interaction (GCI)-GREML approach<sup>15</sup>. They reported significant evidence for G×E  
77 interaction in behavioural phenotypes (education and human reproductive behaviour) and  
78 BMI<sup>15</sup>. The analytical method and designs used in their studies were based on discrete  
79 groups, which ignored the difference of population characteristics for the individuals within  
80 each group. Furthermore, the population groups used in their studies were classified  
81 according to their country origin, thus the results were likely to reflect heterogeneity across  
82 countries due to country-specific factors (e.g., trait definition and measurement<sup>16-18</sup>, cultural  
83 and societal difference and socio-economic status). In addition, genetic measurement errors  
84 (e.g., due to the genotyping platform or imputation quality) across different cohorts may  
85 further cause confounding with genuine genetic heterogeneity across populations<sup>15</sup>.

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87 Principal component (PC) analysis provides a powerful tool to characterize populations and  
88 the first few PCs are typically used to control population stratifications in large-scale GWAS  
89<sup>19</sup>. PCs allow us to cluster individuals that are genetically similar to each other. Unlike  
90 discrete variables such as cohort and country, PCs are continuous variables that can  
91 differentiate individuals even within a cohort or a country according to their underlying  
92 genetic characteristics. Here, we introduce a novel concept of genotype-by-population (G×P)  
93 interaction where population is defined by the first and second PCs. It is of interest to test if  
94 different genotypes respond differently to the gradient of the first or second PC for complex  
95 traits using a whole-genome reaction norm model (RNM)<sup>20</sup>, which has been recently  
96 introduced and allows fitting continuous environmental covariates, i.e. PCs in this study.

97 RNM has been well established to estimate G×E interaction in agriculture<sup>21,22</sup> and ecology<sup>23</sup>.  
98 Furthermore, in this study we used the data source of UK BioBank (UKBB), which is a  
99 prospective cohort study with deep genetic and phenotypic data collected on approximately  
100 500,000 individuals across the United Kingdom, aged between 40 and 69 at recruitment<sup>24,25</sup>.

101 Therefore, in our G×P interaction model applied to UKBB, the population characteristics for  
102 individuals are fully utilised and the findings are less likely to be confounded with country-  
103 specific factors or genetic measurement errors as mentioned above.

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105 The aim of the study is to explore if there exists significant G×P interaction, which is also  
106 referred to genetic heterogeneity (heterogeneous genetic effects) across populations, for a  
107 wide range of complex traits. To do so, we applied the whole-genome RNM with PCs as  
108 continuous covariates to investigate G×P interactions for more than one hundred phenotypes  
109 using the UKBB data. The significant G×P interaction detected in this study may shed light  
110 on the latent genetic architecture of complex traits that underlies the modulation of genetic  
111 effects across different population backgrounds.

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## 113 **Subjects and Methods**

### 114 **Data and quality control (QC)**

115 Our study was based on the UKBB data which contains approximately 500,000 individuals  
116 sampled across the United Kingdom<sup>25</sup>. According to the ethnic background (data field  
117 21000), there are currently 472,242 individuals with the white British ancestry and 17,038  
118 individuals with any other white ethnic background (not with British or Irish ethnicity) in the  
119 UKBB participants. In order to match the sample size between the white British and the other  
120 white ethnic individuals, we randomly selected 17,000 individuals from the white British  
121 group, totalling 34,038 admixed European populations considered in this study. Using  
122 ancestry PCs provided by the UKBB, we examined a two-dimensional scatter plot of the first  
123 and second PC of the 17,000 white British and the 17,038 other white ethnic subjects (Figure  
124 1A). It is shown that the white British group is situated within the group of the other white  
125 Europeans and we named the white British group as POP1 ( $N=17,000$ ). As shown in Figures

126 1B and 1C, we used a geometric method by which we constructed a rectangle with  
127 maximums and minimums of PC1 and PC2 of the white British group as four sides and then  
128 group the individuals of the other white Europeans inside this rectangle, named as POP3  
129 ( $N=9,809$ ). The rest of the other white Europeans except POP3 were named as POP2  
130 ( $N=7,229$ ).

131  
132 Our primary interest was to investigate G×P interaction where population was classified by  
133 ancestry PCs. For this purpose, we used three designs of combinations of the three groups, i.e.  
134 POP1+POP2 (Figure 1B), POP2+POP3 (Figure 1C) and POP1+POP3 (Figure 1D). It was  
135 noted that POP1+POP3 was a negative control as there was little population difference  
136 among them. To make sample size consistent across POP1 and POP2 in the design of  
137 POP1+POP2, we randomly selected 7,500 individuals from the 17,000 white British  
138 individuals and these were used as POP1 in the downstream analyses.

139  
140 We extracted genetic data including around 92 million SNPs from the UKBB for all the  
141 individuals of POP1, POP2 and POP3. Stringent QC was applied to the combined data across  
142 POP1, POP2 and POP3. The QC criteria were to exclude 1) all duplicated and non-autosomal  
143 SNPs, 2) SNPs with INFO score  $< 0.6$ , 3) SNPs with call rate  $< 0.95$ ; (4) individuals with  
144 missing rate  $> 0.05$ , 5) SNPs with Hardy-Weinberg equilibrium  $p$ -value  $< 0.0001$ , 6) SNPs  
145 with minor allele frequency  $< 0.01$ , and 7) SNPs with A/T alleles or G/C alleles. We also  
146 retained HapMap3 SNPs only as they are reliable and robust to bias in estimating SNP-  
147 heritability and genetic correlation<sup>15, 26, 27</sup>. Hereafter, 1,133,957 common SNPs were  
148 remained for the G×P analyses. Moreover, we excluded one individual randomly selected  
149 from any pair with a genetic relationship  $> 0.05$  (see Statistical models) to avoid bias due to

150 confounding by shared environment among close relatives. After the QC, the sample sizes of  
151 POP1, POP2 and POP3 were reduced to 7487, 6913 and 7829.

152

### 153 **UKBB phenotypes**

154 For current UKBB resource, we have access to 496 variables whose data types are categorical  
155 (multiple), categorical (single), continuous, integer, date, text and time. Here we focused on  
156 the variables of categorical (multiple), categorical (single), continuous and integer types, and  
157 categorized each variable as one of four value types: continuous, binary, ordered categorical  
158 and unordered categorical<sup>28</sup> (Table S1). Where a data field is measured at several time points  
159 we use the first occurrence only. It was noted that qualifications (data field 6138), a  
160 categorical (multiple) trait, was reorganised according to the underlying system<sup>29</sup>. Briefly,  
161 the original and unordered seven categories were reclassified and ordered as 1) none, 2) O-  
162 levels or CSEs, 3) A-levels, NVQ, HND, HNC or other professional qualification, and 4)  
163 college or university degree. Then the continuous, binary and ordered categorical variables  
164 were selected and used as the main phenotypes in G×P interaction analyses.

165

166 Since there exist numerous “Not Available” (NA) records for individuals in UKBB, the  
167 limited sample sizes of some variables may lead to insufficient statistical power to perform  
168 our study. Hence, the variables with limited sample size should be excluded. As POP2 and  
169 POP3 have the same ethnic background, we only examined sample sizes of POP1 and POP2,  
170 and used the following thresholds to exclude the variable with: non-NA number in POP1 <  
171 2,500 and non-NA number in POP2 < 2,500, and then we remain 199 variables whose sample  
172 size in POP1+POP2 > 5,000 as shown in Table S1. Note that some ambiguous values in  
173 variables such as “Do not know” or “Prefer not to answer” were treated as NA.

174

175 Among the 199 variables, we selected 128 variables as the main phenotypes (Table S2) in our  
176 proposed model to estimate G×P interactions where population difference was inferred from  
177 the first and second PCs. The other variables were used to control confounding effects owing  
178 to sex, age, year of birth, genotype batch and assessment centre (basic confounders adjusted  
179 for all the main phenotypes; the first 20 PCs were also used as basic confounders to account  
180 for population stratification) and Townsend deprivation index, smoking status, alcohol  
181 consumptions and many other variables (additional cofounders adjusted for some relevant  
182 phenotypes) or excluded if they were not likely to affect any of the main phenotypes (see the  
183 note of Table S2). The 128 main phenotypes could be classified into a number of criteria, 1)  
184 lifestyle and environment (alcohol, diet, electronic device use, sexual factors, sleep, smoking  
185 and sun exposure), 2) physical measures (anthropometry, blood pressure and bone-  
186 densitometry of heel), 3) early life factors, sociodemographics (education, employment and  
187 household), 4) health and medical history (eyesight, hearing, medical conditions and  
188 medication), 5) psychosocial factors (mental health), 6) female-specific factors, male-specific  
189 factors, 7) verbal interview (medical conditions) and 8) cognitive function (reaction time)  
190 (Table S2). Note that some phenotypes such as from sociodemographics (e.g., qualifications)  
191 can also be used as additional confounders for other phenotypes.

192

### 193 **Statistical models**

#### 194 *A linear mixed model without considering G×P interaction (baseline model)*

195 A standard linear mixed model assuming no G×P interaction can be written as

196

$$\mathbf{y} = \boldsymbol{\mu} + \mathbf{g} + \mathbf{e},$$

197 where  $\mathbf{y}$  is an  $n \times 1$  vector of phenotypes with  $n$  being the sample size,  $\boldsymbol{\mu}$  is an  $n \times 1$  vector for

198 fixed effects,  $\mathbf{g}$  is an  $n \times 1$  vector of total genetic effects of the individuals with  $\mathbf{g} \sim N(0, \mathbf{A}\sigma_g^2)$

199 and  $\mathbf{e}$  is an  $n \times 1$  vector of residual effects with  $\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$ , where  $\sigma_g^2$  is the variance



200 explained by all common SNPs and  $\sigma_e^2$  is the residual variance. In the GREML context<sup>30,31</sup>,  
201  $\mathbf{A}$  is a genomic relationship matrix (GRM) and  $\mathbf{I}$  is an identity matrix. GRM can be estimated  
202 based on common SNPs across the genome and the elements of GRM can be defined as<sup>30,32</sup>,  
203 <sup>33</sup>:

$$204 \quad A_{ij} = \frac{1}{L} \sum_{l=1}^L \frac{(x_{il} - 2p_l)(x_{jl} - 2p_l)}{\text{var}(x_l)},$$

205 where  $L$  is the number of all common SNPs ( $L = 1,133,957$  in this study),  $x_{il}$  denotes the  
206 number of copies of the reference allele for the  $l$ th SNP of the  $i$ th individual,  $x_l$  denotes all  
207 the numbers of copies of the reference allele across all the individuals, and  $p_l$  denotes the  
208 reference allele frequency of the  $l$ th SNP.

209  
210 The variance-covariance matrix of the observed phenotypes ( $\mathbf{V}$ ) is

$$211 \quad \mathbf{V} = \mathbf{A}\sigma_g^2 + \mathbf{I}\sigma_e^2.$$

212 The SNP-based heritability, the proportion of the additive genetic variance explained by the  
213 genome-wide SNPs over the total phenotypic variance, is then referred as

$$214 \quad h_{SNP}^2 = \frac{\sigma_g^2}{\sigma_y^2} = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}.$$

215 The phenotypes with significant SNP-based heritability from this baseline model will  
216 subsequently be investigated for G×P interaction.

217

### 218 ***G×P RNM method***

219 In cases where G×P interaction exists across populations, the baseline model cannot account  
220 for heterogeneous genetic effects. We therefore applied RNM methods to detect  
221 heterogeneity across populations using the UKBB data. RNM and multivariate RNM

222 (MRNM) have been demonstrated to perform better than the current state-of-the-art methods  
 223 when detecting genotype-covariate and residual-covariate interactions in terms of simulation  
 224 studies on type I error rate and power analyses<sup>20</sup>. Here we focus on G×P interaction by  
 225 considering PCs as covariates in the RNM:

$$226 \quad \mathbf{y} = \boldsymbol{\mu} + \mathbf{g} + \mathbf{e} = \boldsymbol{\mu} + \mathbf{g}_0 + \mathbf{g}_1 \cdot \mathbf{c} + \mathbf{e},$$

227 where  $\mathbf{y}$ ,  $\boldsymbol{\mu}$ ,  $\mathbf{g}$  and  $\mathbf{e}$  are the same defined in the baseline model above,  $\mathbf{g}_0$  and  $\mathbf{g}_1$  are  $n \times 1$   
 228 vectors of zero- and first-order random regression coefficients, respectively,  $\mathbf{c}$  is an  $n \times 1$   
 229 vector of covariate values of the  $n$  individuals (for which we used PC1 and PC2 values in this  
 230 study). In the RNM, the random genetic effects,  $\mathbf{g}$ , are regressed on the covariate gradient  
 231 (reaction norm), which can be modelled with random regression coefficients,  $\mathbf{g}_0$  and  $\mathbf{g}_1$ .

232 The variance-covariance matrix of the random regression coefficients ( $\mathbf{K}$ ) is

$$233 \quad \mathbf{K} = \begin{pmatrix} \text{var}(\mathbf{g}_0) & \text{cov}(\mathbf{g}_0, \mathbf{g}_1) \\ \text{cov}(\mathbf{g}_0, \mathbf{g}_1) & \text{var}(\mathbf{g}_1) \end{pmatrix}.$$

234 Then the variance-covariance matrix of genetic effects between  $n$  individuals (who have  
 235 unique PC values) can be expressed as

$$236 \quad \mathbf{V}_g = \boldsymbol{\Phi} \mathbf{K} \boldsymbol{\Phi}' = \begin{pmatrix} \sigma_{g(1)}^2 & \cdots & \sigma_{g(1,n)} \\ \vdots & \ddots & \vdots \\ \sigma_{g(n,1)} & \cdots & \sigma_{g(n)}^2 \end{pmatrix},$$

237 where  $\sigma_{g(i)}^2$  denotes the genetic variance at the  $i$ th covariate level,  $\sigma_{g(i,j)}$  indicates the genetic  
 238 covariance between the  $i$ th and  $j$ th covariate levels ( $i = 1, \dots, n$ , and  $j = 1, \dots, n$ ), and

$$239 \quad \boldsymbol{\Phi} = \begin{pmatrix} 1 & c_1 \\ 1 & c_2 \\ \vdots & \vdots \\ 1 & c_n \end{pmatrix} \text{ denotes the covariate matrix. This G} \times \text{P RNM accounts for phenotypic plasticity}$$

240 and norms of reaction in response to different populations (represented by PC values) among  
 241 samples.

242

243 The mathematical properties of  $\mathbf{K}$  allow us to verify whether estimates of the parameters are  
244 reasonable or not. Specifically, estimated values in the matrix  $\mathbf{K}$  should be within a valid  
245 parameter space:

246 (1)  $\text{var}(\hat{\mathbf{g}}_0) \geq 0$ ;

247 (2)  $\text{var}(\hat{\mathbf{g}}_1) \geq 0$ ;

248 (3)  $-\sqrt{\text{var}(\hat{\mathbf{g}}_0) \text{var}(\hat{\mathbf{g}}_1)} \leq \text{cov}(\hat{\mathbf{g}}_0, \hat{\mathbf{g}}_1) \leq \sqrt{\text{var}(\hat{\mathbf{g}}_0) \text{var}(\hat{\mathbf{g}}_1)}$ .

249 The estimates which violated one of above criteria were excluded for follow-up analyses. We  
250 obtained a p-value to detect G×P interaction using a likelihood ratio test (LRT) that compared  
251 the goodness of fitness of two models (GREML and G×P RNM), penalising the difference in  
252 the number of parameters between them.

253

254 We further tested if the significant G×P interactions were orthogonal (independent without  
255 confounding) to residual-population (R×P) interactions, i.e. residual heterogeneity across  
256 populations<sup>20</sup>. Similarly, the R×P interaction can be detected by an R×P RNM:

257 
$$\mathbf{y} = \boldsymbol{\mu} + \mathbf{g} + \mathbf{e} = \boldsymbol{\mu} + \mathbf{g} + \mathbf{e}_0 + \mathbf{e}_1 \cdot \mathbf{c},$$

258 where  $\mathbf{e}_0$  and  $\mathbf{e}_1$  are vectors of zero- and first-order random regression coefficients when  
259 residual effects,  $\mathbf{e}$ , are regressed on the covariate,  $\mathbf{c}$ , i.e. an n vector of PC1 or PC2.

260

261 Furthermore, a full RNM model with both G×P and R×P interactions can be expressed as

262 
$$\mathbf{y} = \boldsymbol{\mu} + \mathbf{g}_0 + \mathbf{g}_1 \cdot \mathbf{c} + \mathbf{e}_0 + \mathbf{e}_1 \cdot \mathbf{c}.$$

263 Since the G×P and R×P models are nested within the full model, LRT comparing the full and  
264 R×P or G×P model with an appropriate degree of freedom can determine the significance of  
265 orthogonal G×P or R×P interaction<sup>20</sup>.

266

267 For the analyses showing a significant G×P interaction, we used rank-based INT phenotypes  
268 to check explicitly if the significance was due to phenotypic heteroscedasticity or normality  
269 assumption violation<sup>34</sup>. The bias of RNM/MRNM estimates due to non-normality of  
270 phenotypic values can also be remedied by applying the rank-based INT<sup>20</sup>. All models  
271 described above (i.e., GREML, bivariate GREML, RNM, MRNM) can be fitted using  
272 software MTG2<sup>33</sup>.

273

### 274 *Spurious signals due to selection or collider bias*

275 We used the UKBB data that have only a 5.5% response rate, i.e. selection. Consequently, the  
276 resulting sample may not be representative of the UK population as a whole and the selection  
277 may be associated with some of the phenotypes in the UKBB, causing selection or collider  
278 bias<sup>35,36</sup>. To test whether the G×P interaction effects detected by our method was genuine or  
279 spurious due to selection or collider bias, we conducted a series of simulation studies with  
280 phenotypes differentially selected for POP1 (white British) and POP2 (other white  
281 Europeans). A selection model using a logistic regression with a trait Y can be written as

$$282 \quad \text{logit}(\mathbf{p}) = \ln\left(\frac{\mathbf{p}}{1-\mathbf{p}}\right) = \boldsymbol{\mu} + \ln(OR_{\text{POP1}, Y}) \cdot \mathbf{y} \quad \text{for POP1}$$

283 and

$$284 \quad \text{logit}(\mathbf{p}) = \ln\left(\frac{\mathbf{p}}{1-\mathbf{p}}\right) = \boldsymbol{\mu} + \ln(OR_{\text{POP2}, Y}) \cdot \mathbf{y} \quad \text{for POP2}$$

285 where  $\mathbf{p}$  is a vector of participation probabilities in a study (e.g., UKBB questionnaire survey)  
286 for all individuals,  $\boldsymbol{\mu}$  is an overall mean vector which regulates the response rate,  $\mathbf{y}$  is a vector  
287 of phenotypic values of the trait Y,  $OR_{\text{POP1}, Y}$  and  $OR_{\text{POP2}, Y}$  are selection odds ratios for  
288 POP1 and POP2, respectively. Then the sample selection bias can be simulated with varying  
289 selection odds ratios.

290

291 One hundred replicates of phenotypic values of the trait  $Y$  on POP1+POP2 (14,400  
292 individuals) were simulated under a baseline model (GREML) that assumes no  $G \times P$   
293 interaction:  $\mathbf{y} = \mathbf{g} + \mathbf{e}$ , where the variance-covariance structure between  $\mathbf{g}$  and  $\mathbf{e}$  was

294  $\begin{pmatrix} 0.5 & 0 \\ 0 & 0.5 \end{pmatrix}$ . For each replicate, to avoid insufficient statistical power, we set  $\boldsymbol{\mu}$  as a vector of

295 zeros which simulates a response rate of 50%. Letting us divide  $\mathbf{y}$  and  $\mathbf{p}$  into subsets

296 according to specific populations (i.e.  $\mathbf{y}_1$  and  $\mathbf{p}_1$  for POP1 and  $\mathbf{y}_2$  and  $\mathbf{p}_2$  for POP2), we

297 obtain the participation probability for each individual as

298 
$$\mathbf{p}_1 = \frac{1}{1 + \exp(-\ln(OR_{POP1,Y}) \cdot \mathbf{y}_1)} \quad \text{for POP1}$$

299 , and

300 
$$\mathbf{p}_2 = \frac{1}{1 + \exp(-\ln(OR_{POP2,Y}) \cdot \mathbf{y}_2)} \quad \text{for POP2.}$$

301 Then, individuals in each population are selected based on the participation probability.

302 Specifically, we generate a uniform distribution vector  $\mathbf{u}_1$  on (0, 1) with sample size of POP1,

303 and compare the values of corresponding components in  $\mathbf{p}_1$  and  $\mathbf{u}_1$ . The individuals having

304 larger values in  $\mathbf{p}_1$  than in  $\mathbf{u}_1$  are assumed to participate in this study. Similarly, we can select

305 individuals in POP2 by comparing  $\mathbf{p}_2$  with a random number drawn from a uniform

306 distribution (0, 1). Different combinations of selection odds ratios for POP1 and POP2 (e.g.,

307  $OR_{POP1,Y} = 1$  and  $OR_{POP2,Y} = 2$ ) will generate selection bias associated with phenotypic

308 values in the POP1+POP2 groups.

309

310 Since the phenotypic data was simulated under the null model, a significant  $G \times P$  interaction

311 detected from LRT comparing  $G \times P$  RNM versus GREML was a type I error (false positive).

312 This allowed us to investigate the type I error rate of G×P interaction due to selection bias  
313 attributed to various selection pressures (odds ratios) on POP1 and POP2. Using the same  
314 simulated data, we also applied a bivariate GREML<sup>37</sup> to test if estimated genetic correlation  
315 between POP1 and POP2 was significantly different from 1 (i.e. evidence of G×P interaction  
316 across POP1 and POP2)<sup>38</sup>. This allowed us to assess the type I error rate of G×P interaction  
317 when using the bivariate GREML.

318

319 If two or more phenotypic variables simultaneously influence the probability of participation  
320 of individuals in a study, then investigating associations between those variables in the  
321 selected sample may induce collider bias<sup>36</sup>. Therefore, we further considered the same  
322 selection model but including two traits to evaluate collider bias effects on the detection of  
323 G×P interaction across POP1 and POP2. The selection model with two traits Y and Z can be  
324 written as

$$325 \quad \text{logit}(\mathbf{p}) = \ln\left(\frac{\mathbf{p}}{1-\mathbf{p}}\right) = \boldsymbol{\mu} + \ln(OR_{\text{POP1},Y}) \cdot \mathbf{y} + \ln(OR_{\text{POP1},Z}) \cdot \mathbf{z} \quad \text{for POP1}$$

326 , and

$$327 \quad \text{logit}(\mathbf{p}) = \ln\left(\frac{\mathbf{p}}{1-\mathbf{p}}\right) = \boldsymbol{\mu} + \ln(OR_{\text{POP2},Y}) \cdot \mathbf{y} + \ln(OR_{\text{POP2},Z}) \cdot \mathbf{z} \quad \text{for POP2}$$

328 where  $\mathbf{z}$  is a vector of phenotypic values of the trait Z,  $OR_{\text{POP1},Z}$  and  $OR_{\text{POP2},Z}$  are selection  
329 odds ratios with the trait Z for POP1 and POP2. The magnitude of collider bias depends on  
330 the levels of selection odds ratios for the two phenotypes.

331

332 We simulated 100 replicates of phenotypic values of the trait Z on POP1+POP2 under the  
333 null model of no G×P interaction:  $\mathbf{z} = \boldsymbol{\alpha} + \boldsymbol{\beta}$ , where the variance-covariance structure between

334  $\boldsymbol{\alpha}$  and  $\boldsymbol{\beta}$  is  $\begin{pmatrix} 0.5 & 0 \\ 0 & 0.5 \end{pmatrix}$ . Since genetic components  $\mathbf{g}$  and  $\boldsymbol{\alpha}$  are uncorrelated and residual

335 components  $\mathbf{e}$  and  $\boldsymbol{\beta}$  are uncorrelated, the phenotypic variable  $\mathbf{z}$  and previous simulated  $\mathbf{y} = \mathbf{g}$   
336 +  $\mathbf{e}$  are totally independent, but after selection we expect that the two variables will be  
337 associated because of a collider. Letting us divide  $\mathbf{z}$  into subsets according to specific  
338 populations (i.e.  $\mathbf{z}_1$  for POP1 and  $\mathbf{z}_2$  for POP2), the individuals can be selected based on

$$339 \quad \mathbf{p}_1 = \frac{1}{1 + \exp(-\ln(OR_{POP1,Y}) \cdot \mathbf{y}_1 - \ln(OR_{POP1,Z}) \cdot \mathbf{z}_1)} \quad \text{for POP1}$$

340 and

$$341 \quad \mathbf{p}_2 = \frac{1}{1 + \exp(-\ln(OR_{POP2,Y}) \cdot \mathbf{y}_2 - \ln(OR_{POP2,Z}) \cdot \mathbf{z}_2)} \quad \text{for POP2.}$$

342 Similarly, we can select individuals in POP1 or POP2 by comparing  $\mathbf{p}_1$  or  $\mathbf{p}_2$  with a random  
343 number drawn from a uniform distribution (0, 1). Therefore, in terms of collider bias,  
344 different combinations of selection odds ratios for different traits and populations (e.g.,  
345  $OR_{POP1,Y} = 2$ ,  $OR_{POP2,Y} = 3$ ,  $OR_{POP1,Z} = 2$  and  $OR_{POP2,Z} = 2$ ) will generate collider bias in the  
346 POP1+POP2 groups. Similarly, we can examine G×P interaction by type I error rate analysis  
347 using G×P RNM and bivariate GREML methods, and assess collider bias effects for the two  
348 methods.

349

## 350 **Results**

### 351 **Estimating SNP-based heritability for 128 phenotypes**

352 We first applied the standard GREML model to estimate  $h_{SNP}^2$  for the 128 phenotypes across  
353 POP1+POP2, POP2+POP3 and POP1+POP3, respectively. The phenotypes with significant  
354  $h_{SNP}^2$  (Tables S3-5) were further investigated for G×P interaction effects using our G×P RNM  
355 approach.

356

### 357 **Genetic and residual correlations between phenotypes and PCs**

358 The main response ( $\mathbf{y}$ ) and environmental covariates ( $\mathbf{c}$ ) are not always uncorrelated, for  
359 which multivariate RNM accounting for (genetic and residual) correlations between  $\mathbf{y}$  and  $\mathbf{c}$   
360 should be used<sup>20</sup>. We examined if there were non-negligible genetic and residual covariances  
361 between the main phenotypes and covariate (PC1 or PC2) for the complex traits with  
362 significant heritabilities (Tables S6-8). All genetic and residual covariances estimated by  
363 bivariate GREML were not significantly different from zero, and thus we used univariate  
364 RNM to detect the G×P interaction effects with covariate PC1/PC2 for those phenotypes.

365

### 366 **G×P interaction**

367 For POP1+POP2, we fit the data of the 70 phenotypes with significant  $h_{SNP}^2$  by modelling the  
368 G×P RNM with covariates PC1 and PC2, respectively (Tables S9 and S10). We excluded  
369 those estimates, which were not within the valid parameter space (see Statistical models),  
370 from the follow-up statistical test analyses, resulting in 29 and 32 traits remaining for PC1  
371 (Table S9) and PC2 analyses (Table S10). We examined if there was significant G×P  
372 interaction and obtained p-values based on LRT comparing the fit to the data of the G×P  
373 RNM and null model. Significance level was determined by Bonferroni multiple testing  
374 correction:  $0.05/140 = 3.57E-4$  for the 70 phenotypes with covariates PC1 and PC2. Figures  
375 S1A and S1B show that significant G×P interactions were found for ten complex traits which  
376 are related to blood pressure (pulse rate, automated reading), bone-densitometry of heel (heel  
377 BMD T-score, automated; heel broadband ultrasound attenuation, direct entry; heel QUI,  
378 direct entry; heel BMD), diet (lamb/mutton intake), sexual factor (age first had sexual  
379 intercourse), sleep (sleep duration), smoking (ever smoked) and education (qualifications).  
380 For each of the ten traits, we further considered a multiple covariate model that fit PC1 and  
381 PC2 jointly (Table S11). However, G×P interactions were less significant than those obtained  
382 using the single covariate model fitting PC1 or PC2 separately (Figure S2), otherwise, the



383 estimates were out of the valid parameter space. This was probably due the fact that there was  
384 collinearity between G×P interactions from PC1 and PC2.

385

386 In addition to the basic confounders for which the main phenotypes were initially adjusted  
387 (see Subjects and Methods), we further considered additional trait-specific confounders that  
388 might be relevant to some of traits (Table S2), e.g. Townsend deprivation index, smoking  
389 status, alcohol drinker status, etc. After controlling for additional trait-specific confounders,  
390 the G×P interactions in POP1+POP2 were still significant for bone-densitometry of heel (heel  
391 BMD T-score, automated; heel broadband ultrasound attenuation, direct entry; heel QUI,  
392 direct entry; heel BMD), age first had sexual intercourse and qualifications, whereas the  
393 signals disappeared for the other traits (Table S12).

394

395 We examined the distribution of phenotypic values after controlling additional confounders  
396 of the six traits with significant G×P interactions (Figure S3) and could not rule out the  
397 possibility that the interaction signals were due to non-normality (e.g. residual  
398 heteroscedasticity). We conducted the same analyses for the six traits using rank-based INT  
399 phenotypes (Table 1), which could control type I error rate due to a skewed and non-normal  
400 distribution of residual values<sup>20</sup>. Indeed, phenotypic heteroscedasticity was remedied when  
401 using rank-based INT for the phenotypes of six traits as shown in Figures S4-9. We found  
402 that the interaction signals of age first had sexual intercourse and qualifications were  
403 remained significant even after applying rank-based INT phenotypes, however, the other  
404 traits were not significant anymore (Table 1).

405

406 For age first had sexual intercourse and qualifications that were shown to have significant  
407 G×P interactions, we further tested if the G×P interactions were orthogonal to R×P

408 interactions, i.e. residual heterogeneity (see Subjects and Methods). Using the rank-based  
409 INT phenotypes adjusted for basic and additional confounders, we carried out an R×P model  
410 and a full model in which both G×P and R×P were fitted jointly. Subsequently, we conducted  
411 LRT to obtain p-values, comparing the full and nested models. A significant p-value from  
412 LRT between the full and R×P model indicates that G×P interaction is orthogonal to R×P  
413 interaction (see Subjects and Methods and Tables S13). For age first had sexual intercourse,  
414 although G×P or R×P interaction was significantly detected from the G×P or R×P model, it  
415 was shown that G×P interaction was not orthogonal to R×P (p-value = 0.88 for PC1 and 0.92  
416 for PC2 in Table S13). For qualifications, on the other hand, it was shown that the G×P and  
417 R×P interactions were statistically independent (p-value = 4.15E-05 for PC1 and 0.003 for  
418 PC2 in Table S13).

419  
420 For POP2+POP3, we conducted analyses using the same procedure as in the analyses of  
421 POP1+POP2. The POP3 individuals are very close to those in POP1 in terms of ancestry PC,  
422 but their ethnicities are not white British as in POP1 (see Subjects and Methods and Figure 1).  
423 Thirteen phenotypes demonstrated a significant genetic heterogeneity for covariate PC1 or  
424 PC2 as shown in Tables S14 and S15. After controlling for additional trait-specific  
425 confounders and transforming by rank-based INT (Table S16), the results for behavioural  
426 phenotypes age first had sexual intercourse (p-value = 7.86E-05 for PC1) and qualifications  
427 (p-value = 1.06E-15 for PC1) have demonstrated strong genetic heterogeneity signals, which  
428 are consistent with our findings for POP1+POP2. For qualifications, G×P interactions were  
429 significantly orthogonal to R×P interactions (p-value = 0.003 for PC1 in Table S17). We also  
430 found significant results across POP2+POP3 for anthropometric traits (waist circumference  
431 and weight) and diabetes diagnosed by doctor. However, these phenotypes were not  
432 discovered across POP1+POP2 with significant G×P interaction signals. We presented

433 genetic variance, interaction variance and their covariance component estimates for these  
434 significant traits across POP2+POP3 in Table 2.

435

436 We also performed the same analyses on POP1+POP3, which is not a diverse population  
437 group as POP1+POP2 or POP2+POP3, and thus was used as a negative control group (see  
438 Methods). For several traits showing significant heterogeneous signals with covariate PC1 or  
439 PC2 after Bonferroni correction (see Tables S18 and S19), we further examined them by  
440 adding stringent confounders to correct for fixed effects and applying rank-based INT. The  
441 final results included no significant G×P interaction across POP1+POP3 (see Tables S20 and  
442 S21).

443

444 For the categorical phenotype qualifications, there were various ways to convert the seven  
445 UKBB categories into a continuous or a binary measure<sup>39,40</sup>. Following a previous study<sup>39</sup>,  
446 we transformed the multiple categories (data fields: 6138.0.0 to 6138.0.5) into an educational  
447 year measure (Table S22). Based on this continuous phenotypic measure, we found  
448 significant genetic heterogeneity across POP1+POP2 and POP2+POP3 but no signal across  
449 POP1+POP3 (Table S23), which was consistent with our results obtained using four-level  
450 categories. We also examined G×P interactions for qualifications based on two types of  
451 binary measures (highest educational attainment versus other levels, and lowest educational  
452 attainment versus other levels)<sup>40</sup>. The results were consistent with those obtained using four-  
453 level qualifications, except that an unexpected significant signal across POP1+POP3 for  
454 covariate PC1 was detected based on the binary measure of “college or university degree”  
455 versus other six categories (Table S24).

456

457 **Testing effects of selection or collider bias**

458 We examined the distribution of phenotypic values for age first had sexual intercourse and  
459 qualifications in which G×P interactions were consistently detected from both POP1+POP2  
460 and POP2+POP3 (Tables S25 and S26). The distribution of age first had sexual intercourse is  
461 similar across POP1, POP2 and POP3. However, for qualifications, it is apparently shown  
462 that the subjects in POP2 and POP3 (other white Europeans) have higher qualification levels  
463 than those in POP1 (white British). Moreover, it is likely that the individuals in POP1 have  
464 higher educational levels than the general population of UK because individuals with higher  
465 educational levels are more likely to response to surveys from UKBB <sup>36</sup>.

466

467 Our simulation studies testing for detecting spurious heterogeneity across POP1 and POP2  
468 with multiple scenarios varying the level of selection odds ratios (see Supplementary Notes  
469 for details) have verified that (1) both G×P RNM and bivariate GREML are robust to the  
470 selection bias when using the same selection odds ratio across populations (Table 3); (2) only  
471 bivariate GREML is robust against the selection bias when using different selection odds  
472 ratios across populations (Table 3); (3) bivariate GREML is robust against the collider bias  
473 when estimating genetic correlation between POP1 and POP2, however it generates biased  
474 estimation of genetic correlation between the two traits (Table 4). It is noted that the level of  
475 selection odds ratios used in simulations is likely to reflect the real situation of qualifications,  
476 i.e. different selection pressure between POP1 and POP2 in UKBB (see Supplementary Notes  
477 and Tables S27).

478

479 For age first had sexual intercourse and qualifications, we confirmed our findings using  
480 bivariate GREML, a robust approach against selection bias (Table 5). The bivariate GREML  
481 results for qualifications indicated a significant genetic heterogeneity between POP1 and  
482 POP2 (p-value = 8.09E-04), and between POP2 and POP3 (p-value = 7.85E-04), but showed

483 no genetic heterogeneity between POP1 and POP3. These results were consistent with our  
484 findings from the G×P RNM. For age first had sexual intercourse, the bivariate GREML  
485 detected a significant heterogeneity between POP2 and POP3 (p-value = 3.14E-05), however,  
486 there was no interaction signal between POP1 and POP3 (as expected). Unexpectedly, the  
487 bivariate GREML failed to find genetic heterogeneity across POP1+POP2 (Table 5) although  
488 RNM provided a significant signal.

489

490 As confirmed by the bivariate GREML, it was not likely that the findings for qualifications  
491 were spurious because of selection and collider bias. This was also evidenced by the fact that  
492 G×P RNM detected a significant interaction signal from POP2+POP3, noting that POP2 and  
493 POP3 were similarly distributed for qualifications (see Table S26). Similarly, the findings for  
494 age first had sexual intercourse were mostly robust whether using RNM or bivariate GREML  
495 except that there was no signal for POP1+POP2 when using the bivariate GREML, probably  
496 due to the lack of power. It was noted that the phenotypic distributions of age first had sexual  
497 intercourse were very similar across POP1, POP2 and POP3 (Table S25).

498

#### 499 **Hidden heritability**

500 For the significant traits qualifications and age first had sexual intercourse, we examined  
501 SNP-based heritabilities estimated by GREML and G×P RNM (see Table S28). The  
502 phenotypic values were adjusted for basic and additional confounders of fixed effects and  
503 transformed using rank-based INT. For POP1+POP2, the SNP-based heritability for  
504 qualifications estimated by G×P RNM increased by 28% (from 0.0998 to 0.1281) and 84%  
505 (from 0.0998 to 0.1840) with covariate PC1 and PC2, compared to those estimated by  
506 GREML. But there was no such apparent increase of estimated SNP-based heritability for  
507 POP2+POP3 and POP1+POP3 when comparing GREML and G×P RNM.

508

## 509 **Discussion**

510 Previous results<sup>12-15</sup> were more likely to reflect heterogeneous genetic effects across nations  
511 or cohorts rather than populations as those designs were evidently confounded with country-  
512 specific factors (e.g., trait definition and measurement, cultural and societal difference). In  
513 this study, we focused on populations and proposed the new concept “genotype-population  
514 interaction” in which population is defined by the first and second ancestry PCs (each  
515 individual has a unique PC value). Using the RNM with whole-genome data from the UKBB,  
516 we have demonstrated significant G×P interaction effects for qualifications and age first had  
517 sexual intercourse across populations. Our findings corroborate the results in Tropf et al.<sup>15</sup>  
518 who reported that behavioural phenotypes (education and human reproductive behaviour)  
519 have significant G×E interactions across populations. For anthropometric phenotypes, height  
520 and BMI, our G×P RNM model did not detect any significant interaction signals<sup>14</sup>. However,  
521 the analyses of another two anthropometric traits (waist circumference and weight) have  
522 demonstrated significant genetic heterogeneity across the POP2+POP3 group (other white  
523 Europeans). Actually, the results by Tropf et al.<sup>15</sup> across seven populations have also  
524 revealed significant G×E interaction for BMI although the heterogeneity is not strong as for  
525 education and reproductive behaviours. Robinson et al.<sup>12</sup> also reported that, for BMI,  
526 environmental differences across Europe masked genetic differentiation. Thus, these findings  
527 may be consistent for some anthropometric phenotypes when using diverse European  
528 ancestry populations. From the POP2+POP3 analyses, we also found a significant G×P  
529 interaction for diagnosis of diabetes that is a binary response variable.

530

531 As the RNM has not been explicitly verified for binary traits, we also used bivariate GREML  
532 to estimate the genetic correlation between POP2 and POP3 for this disease trait and found

533 no significant signal for genetic heterogeneity (estimate is 0.7988, SE = 0.2044, p-value =  
534 0.3249). This might be due to the fact that there was no genuine interaction effects or that the  
535 bivariate GREML was simply underpowered. For the two binary measuring ways of  
536 qualifications (lowest educational attainment versus other levels, and highest educational  
537 attainment versus other levels), we also used bivariate GREML to examine genetic  
538 correlations between POP1, POP2 and POP3 (Table S29). The results for the binary  
539 phenotype of “none of the above” versus other six educational categories demonstrated  
540 significant genetic heterogeneity between POP1 and POP2 (p-value = 5.58E-05) and between  
541 POP2 and POP3 (p-value = 7.59E-05) but no significant signal between POP1 and POP3 (p-  
542 value = 0.0619), which were consistent with those obtained from the main analyses. For the  
543 binary data of “college or university degree” versus other six categories, the bivariate  
544 GREML indicated a marginally significant heterogeneity between POP1 and POP2 (p-value  
545 = 0.035) and no significant signal between POP2 and POP3 (p-value = 0.494), and POP1 and  
546 POP3 (p-value = 0.94). The reason that the genetic heterogeneity became weaker or  
547 disappeared is probably due to the fact that the bivariate GREML has less power compared to  
548 the RNM approach, and the phenotype categories reduced from four to two levels.  
549  
550 Our results imply that causal variants at multiple loci may not be universal but rather specific  
551 to populations for some complex traits. The results on qualifications across POP1+POP2  
552 suggested that G×P interaction might be a reason for attenuation of SNP-based heritability  
553 when using data from different populations, for which we hold the same view as by Tropf et  
554 al.<sup>15</sup>. This missing or hidden heritability issue<sup>41</sup> can produce lower predictive power of  
555 polygenic risk scores from large GWAS (usually generated from meta-analyses of different  
556 populations) compared with single homogenous population since the reference heritability  
557 obtained from the meta-analyses among several populations is smaller than that obtained

558 from single homogenous population<sup>42</sup>. Therefore, our findings suggest that large  
559 homogeneous population data sources (e.g., around 400,000 white British individuals in the  
560 UKBB) should be used to conduct genetic risk prediction for some specific traits such as  
561 human behaviors.

562

563 The current methods used for estimating G×E (or G×P) interactions, e.g. random regression  
564 (RR)-GREML<sup>22</sup> and GCI-GREML<sup>12,15</sup>, require that the main response should be stratified  
565 into multiple discrete groups according to covariate levels even for a continuous covariate<sup>13</sup>.  
566 However, the arbitrary grouping ignores the difference of covariate values for the individuals  
567 within each group, and results in some loss of information. In contrast, the RNM allows us to  
568 fit a continuous covariate representing individuals uniquely (e.g. PC) in the model and  
569 produces unbiased estimates<sup>20</sup>. In our results, bivariate GREML which labels the individuals  
570 into two discrete groups (POP1 and POP2) failed to find genetic heterogeneity for age first  
571 had sexual intercourse (Table S27), while RNM detected significant G×P interaction across  
572 POP1+POP2 (see Table 1). It may imply that G×P RNM is more powerful as it uses  
573 individual-level information represented by PC across populations, while bivariate GREML  
574 ignores such information within each stratified group. However, on the other hand, RNM  
575 may suffer from the selection bias when using different selection odds ratios across  
576 populations (Table 3) while bivariate GREML is robust against such selection and collider  
577 bias (Tables 3 and 4).

578

579 Residual-covariate interaction may result in heterogeneous residual variances across different  
580 covariate values, thus it is necessary to examine and distinguish genotype-covariate and  
581 residual-covariate interactions<sup>20</sup>. Our results (Tables S13 and S17) provided cogent evidence  
582 of G×P and R×P interaction effects, which are (partially) independent without confounding,



583 across populations for qualifications. However, for age first had sexual intercourse, there was  
584 no evidence showing that G×P interaction was orthogonal to R×P interaction from LRT  
585 comparing the full and nested models. Therefore, we could not rule out the possibility that the  
586 significant signal was mainly because of residual heterogeneity across populations. In order  
587 to disentangle G×P interaction from R×P interaction, the magnitude of G×P interaction  
588 should be large (e.g. qualifications) or sample size may have to be increased.

589

590 The previous results <sup>12-15</sup> were based on pooled data across different nations, and thus various  
591 trait definitions in phenotypic measure and genetic measurement errors across countries may  
592 generate artificial heterogeneity. In our study, however, we used the data resource  
593 standardized across one country (the United Kingdom) to rule out those cross-country factors  
594 and influences. The phenotypic definitions and measurement of complex traits in this cohort  
595 have been standardized nationwide. Moreover, UKBB utilized uniform standards of  
596 imputation and quality control for genotype data and provided genotyping batch information  
597 for each individual that was used as fixed effect adjusted in our models. Therefore, our results  
598 may reflect authentic G×P interaction effects across populations.

599

600 There are several limitations in this study. Firstly, we examined G×P interaction across  
601 populations using three data designs (POP1+POP2 and POP2+POP3 as primary data, and  
602 POP1+POP3 as a negative control), in which population is referred to the first and second  
603 ancestry PCs. As POP1 and POP3 are very close in terms of PCs, the individuals in the two  
604 primary groups POP1+POP2 and POP2+POP3 have common population structures (Figure  
605 1). But both groups involve in different white ethnic backgrounds, i.e., POP1 may be closer  
606 to native British and POP2/POP3 is more likely to be descended from recent immigrants from  
607 many other European nations. Therefore, for our data designs, we cannot rule out the

608 possibility that G×P interaction was confounded with immigration-specific factors such as  
609 socioeconomic attainment, social relations and cultural beliefs<sup>43</sup>. We also notice that, in the  
610 UKBB data source, there are numerous samples with other ethnicities (e.g., Indian, Caribbean  
611 and African), thus future studies using our approach may aim to detect genotype-ethnicity  
612 interaction, which may uncover challenges for investigations into the genetic architecture of  
613 phenotypes across various ethnicities. Secondly, population defined by PCs in this study or  
614 by discrete groups in others<sup>14, 15</sup> includes both environmental and genetic information for  
615 individuals, thus the G×P interaction may not merely embody G×E interaction but also  
616 contains confounded genotype-by-genotype (G×G) interaction across populations. It may  
617 become a new challenge in the future to distinguish G×E and G×G in studies of genetic  
618 heterogeneity across populations. Thirdly, the sample size for people with other white  
619 ethnicity in UKBB (i.e., the sum of POP2 and POP3) is not large, thus the study may lack  
620 power for phenotypes with small SNP-based heritability such as behavioural traits. The  
621 phenotypes without significant heritability in the current samples were not investigated for  
622 G×P interaction, however, if boosting statistical power for those phenotypes, there may be  
623 new findings for heterogeneity across populations. Fourthly, the simulations on selection bias  
624 have demonstrated that the G×P RNM is not robust for data across populations with different  
625 selection odds ratios (see Table 3). Thus our approach is more preferable and restricted to  
626 data without selection bias or with the same selection pressure for populations. Finally, for  
627 genotypic information used in this study, we only examined common SNPs (minor allele  
628 frequency > 0.01). However, a recent study<sup>44</sup> reported that the missing heritability for height  
629 and BMI may be explained by rare genetic variants accessed from whole-genome sequence  
630 data. Therefore, can rare population-specific variants increase our understanding of genetic  
631 heterogeneity across populations? Further research is required to answer this question.

632

633 In conclusion, our study provided a paradigm shift tool in investigating genetic heterogeneity  
634 across populations. The new concept of G×P interaction with the use of ancestry PC is more  
635 plausible in explaining the genetic architecture of complex traits across heterogeneous  
636 populations. The G×P interaction effects on behavioural phenotypes (qualifications and age  
637 first had sexual intercourse) were found by a powerful approach based on technically  
638 homogeneous data (free of genetic measurement errors and cohort/country confounding  
639 factors), and these findings were validated in both data designs POP1+POP2 and  
640 POP2+POP3. The analyses performed in this study can be applied to dissect the genetic  
641 architecture of complex traits and diseases across populations, and the results from these  
642 analyses will provide important information and suggestion for studies of genomic risk  
643 prediction across Europeans.

644

## 645 **Supplemental Data**

646 Supplemental data file includes supplemental notes, 9 figures and 29 tables and can be found  
647 with this article online.

648

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655

## 656 **Declaration of Interests**

657 The authors declare no conflict of interest.

658

## 659 **Web Resources**

660 UK Biobank, <http://www.ukbiobank.ac.uk>

661 PLINK v1.90, <https://www.cog-genomics.org/plink2>

662 MTG2, <https://sites.google.com/site/honglee0707/mtg2>

663

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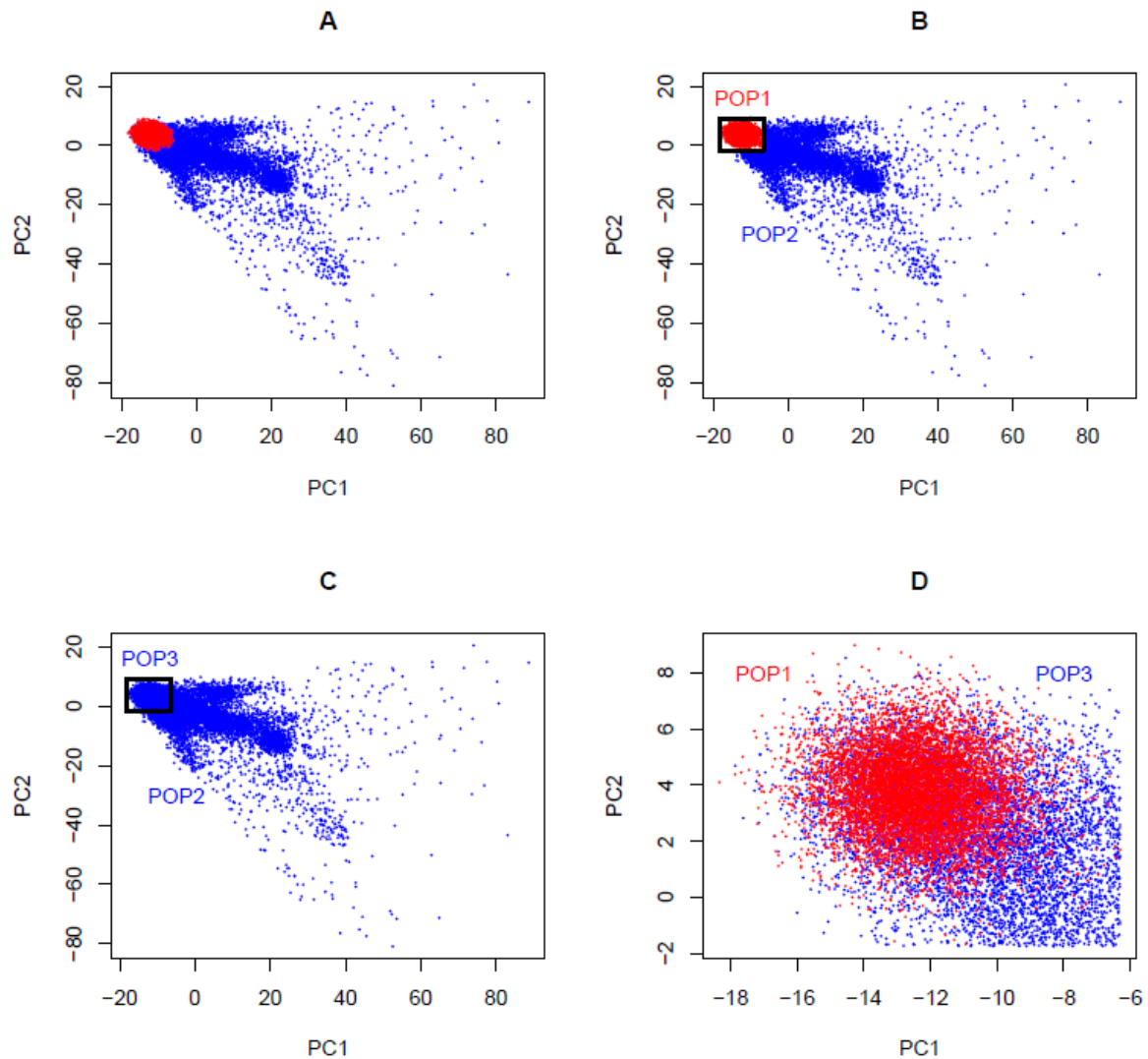
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801 **FIGURE TITLES AND LEGENDS**



802

803 **Figure 1. Two-dimensional scatter plots of PC1 and PC2 with red points representing**  
804 **white British individuals and blue points representing other white ethnic individuals**  
805 **from the UKBB.**

806 The white British group named as POP1 is situated within the group of the other white  
807 Europeans. As shown in (B) and (C), we used a geometric method by which we constructed a  
808 rectangle with maximums and minimums of PC1 and PC2 of POP1 as four sides and then  
809 group the individuals of the other white Europeans inside this rectangle, named as POP3. The  
810 rest of the other white Europeans except POP3 were named as POP2.

## TABLE TITLES AND LEGENDS

**Table 1. Genetic variance, interaction variance and their covariance component estimates for six phenotypes across POP1+POP2 with the covariates PC1 and PC2.** The phenotypes were adjusted by basic plus additional confounders of fixed effects and transformed by rank-based INT. The estimates which were not within the valid parameter space are marked as “Excluded”. SE denotes standard error. DF denotes degree of freedom.

UKBB data field	Phenotype	Covariate	$\text{var}(\mathbf{g}_0)$ (SE)	$\text{var}(\mathbf{g}_1)$ (SE)	$\text{cov}(\mathbf{g}_0, \mathbf{g}_1)$ (SE)	$\text{var}(\mathbf{e}_0)$ (SE)	<i>P</i> -value by LRT comparing with baseline model (DF = 2)
78	Heel bone mineral density (BMD) T-score, automated	PC1	0.3151(0.0459)	0.0124(0.0110)	0.0013(0.0120)	0.6739(0.0456)	0.1586
		PC2	0.3187(0.0460)	-0.0008(0.0047)	-0.0037(0.0110)	0.6838(0.0450)	Excluded
3144	Heel Broadband ultrasound attenuation, direct entry	PC1	0.2754(0.0454)	0.0094(0.0110)	0.0087(0.0120)	0.7161(0.0454)	0.0789
		PC2	0.2774(0.0454)	0.0006(0.0048)	-0.0024(0.0111)	0.7232(0.0450)	0.8987
3147	Heel quantitative ultrasound index (QUI), direct entry	PC1	0.3151(0.0459)	0.0124(0.0110)	0.0013(0.0120)	0.6739(0.0456)	0.1597
		PC2	0.3187(0.0460)	-0.0009(0.0047)	-0.0037(0.0110)	0.6839(0.0450)	Excluded
3148	Heel bone mineral density (BMD)	PC1	0.3070(0.0458)	0.0107(0.0109)	0.0046(0.0120)	0.6836(0.0455)	0.1315
		PC2	0.3106(0.0459)	-0.0016(0.0046)	-0.0069(0.0110)	0.6926(0.0450)	Excluded
2139	Age first had sexual intercourse	PC1	0.1006(0.0266)	0.0080(0.0078)	0.0203(0.0087)	0.8909(0.0290)	5.16E-05
		PC2	0.1012(0.0266)	0.0110(0.0057)	-0.0015(0.0087)	0.8880(0.0286)	0.0097
6138	Qualifications	PC1	0.1194(0.0235)	0.0706(0.0103)	-0.0791(0.0090)	0.8124(0.0261)	9.21E-18
		PC2	0.1778(0.0214)	0.0360(0.0059)	0.0833(0.0081)	0.7885(0.0233)	2.22E-24

**Table 2. Genetic variance, interaction variance and their covariance component estimates for six phenotypes across POP2+POP3 with the covariates PC1 and PC2.** The phenotypes were adjusted by basic plus additional confounders of fixed effects and transformed by rank-based INT. SE denotes standard error. DF denotes degree of freedom.

UKBB data field	Phenotype	Covariate	$\text{var}(\mathbf{g}_0)$ (SE)	$\text{var}(\mathbf{g}_1)$ (SE)	$\text{cov}(\mathbf{g}_0, \mathbf{g}_1)$ (SE)	$\text{var}(\mathbf{e}_0)$ (SE)	<i>P</i> -value by LRT comparing with baseline model (DF = 2)
48	Waist circumference	PC1	0.1802(0.0243)	0.0222(0.0069)	-0.0395(0.0079)	0.7990(0.0256)	2.92E-06
		PC2	0.1789(0.0243)	0.0076(0.0037)	0.0300(0.0078)	0.8147(0.0252)	0.0004
21002	Weight	PC1	0.2537(0.0252)	0.0209(0.0069)	-0.0328(0.0081)	0.7270(0.0257)	0.0002
		PC2	0.2529(0.0252)	0.0077(0.0040)	0.0219(0.0080)	0.7408(0.0252)	0.0252
2443	Diabetes diagnosed by doctor	PC1	0.1688(0.0203)	0.0259(0.0070)	-0.0015(0.0077)	0.7901(0.0218)	6.65E-11
		PC2	0.1734(0.0204)	0.0162(0.0051)	-0.0005(0.0076)	0.7966(0.0219)	3.73E-08
2139	Age first had sexual intercourse	PC1	0.0936(0.0258)	0.0267(0.0086)	-0.0072(0.0087)	0.8795(0.0283)	7.86E-05
		PC2	0.0933(0.0258)	0.0153(0.0056)	0.0112(0.0086)	0.8918(0.0278)	0.0071
6138	Qualifications	PC1	0.0937(0.0264)	0.0324(0.0094)	0.0159(0.0091)	0.8715(0.0287)	1.06E-15
		PC2	0.1139(0.0267)	0.0150(0.0057)	0.0137(0.0086)	0.8713(0.0285)	0.0162

**Table 3. Simulation study results for selection bias on the phenotype Y across POP1+POP2.** Different odds ratio combinations ( $OR_{POP1,Y}$  and  $OR_{POP2,Y}$ ) generated phenotypic values in POP1+POP2 with different selection bias levels. Type I error rates based on 100 simulation replicates were examined by G×P RNM and bivariate GREML respectively. The genetic correlations of the phenotype between POP1 and POP2 were estimated by bivariate GREML. SE denotes standard error.

Selection scenarios in POP1+POP2	Type I error rate by G×P RNM with PC1	Type I error rate by bivariate GREML	100 estimated genetic correlations	
			Mean	SE
$OR_{POP1,Y} = 1, OR_{POP2,Y} = 1$	5%	0%	0.9722	0.0145
$OR_{POP1,Y} = 1, OR_{POP2,Y} = 2$	55%	2%	0.9876	0.0166
$OR_{POP1,Y} = 2, OR_{POP2,Y} = 2$	1%	0%	1.0245	0.0160
$OR_{POP1,Y} = 2, OR_{POP2,Y} = 3$	64%	6%	0.9882	0.0202

**Table 4. Simulation study results for collider bias on two phenotypes Y and Z across POP1+POP2.** Different odds ratio combinations ( $OR_{POP1,Y}$ ,  $OR_{POP2,Y}$ ,  $OR_{POP1,Z}$  and  $OR_{POP2,Z}$ ) generated phenotypes in POP1+POP2 with different selection bias levels. Type I error rates based on 100 simulation replicates were examined through estimated genetic correlations of the phenotype Y between POP1 and POP2 by bivariate GREML. SE denotes standard error.

Selection scenarios with collider bias in POP1+POP2	Type I error rate	Estimated genetic correlations of the phenotype Y between POP1 and POP2		Estimated genetic correlations between Y and Z on selected POP1+POP2	
		Mean	SE	Mean	SE
$OR_{POP1,Y} = 2, OR_{POP1,Z} = 2,$ $OR_{POP2,Y} = 3, OR_{POP2,Z} = 2$	1%	1.0141	0.0189	-0.2516	0.0032
$OR_{POP1,Y} = 2, OR_{POP1,Z} = 2,$ $OR_{POP2,Y} = 3, OR_{POP2,Z} = 3$	2%	1.0220	0.0165	-0.2942	0.0031
$OR_{POP1,Y} = 2, OR_{POP1,Z} = 3,$ $OR_{POP2,Y} = 3, OR_{POP2,Z} = 3$	2%	1.0091	0.0187	-0.3415	0.0036

**Table 5. Genetic correlation estimates between population groups (POP1, POP2 and POP3) by bivariate GREML for two phenotypes.** Here the phenotypes were adjusted by basic plus additional confounders of fixed effects and transformed by rank-based INT. SE denotes standard error. P-value was obtained through a Wald test under a null hypothesis that genetic correlation equals to 1.

Phenotype	Genetic correlation between POP1 and POP2			Genetic correlation between POP2 and POP3			Genetic correlation between POP1 and POP3		
	Estimate	SE	P value	Estimate	SE	P value	Estimate	SE	P value
Qualifications	0.2554	0.2223	8.09E-04	0.4795	0.1550	7.85E-04	0.5676	0.2743	0.1149
Age first had sexual intercourse	0.7418	0.3984	0.5169	0.0491	0.2284	3.14E-05	1.2176	0.3629	0.5488