

# 1 Population stratification in GWAS meta-analysis should be 2 standardized to the best available reference datasets

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## 12 Abstract

13 Population stratification has recently been demonstrated to bias genetic studies even in relatively  
14 homogeneous populations such as within the British Isles. A key component to correcting for  
15 stratification in genome-wide association studies (GWAS) is accurately identifying and controlling for  
16 the underlying structure present in the sample. Meta-analysis across cohorts is increasingly important  
17 for achieving very large sample sizes, but comes with the major disadvantage that each individual  
18 cohort corrects for different population stratification. Here we demonstrate that correcting for  
19 structure against an external reference adds significant value to meta-analysis. We treat the UK  
20 Biobank as a collection of smaller studies, each of which is geographically localised. We provide  
21 software to standardize an external dataset against a reference, provide the UK Biobank principal  
22 component loadings for this purpose, and demonstrate the value of this with an analysis of the  
23 geographically sampled ALSPAC cohort.

## 24 Introduction

25 Genome-wide association studies (GWAS) are increasingly being used to identify biological pathways  
26 underlying complex traits and diseases. They have become an essential part of making direct links  
27 between genetics and phenotypes (Visscher et al. 2017) and have facilitated causal inference  
28 through Mendelian Randomization (Paternoster, Tilling, and Smith 2017; Zhu et al. 2018). However,  
29 detecting and interpreting associations remains a challenge because genetic associations tend to be  
30 tiny (particularly for polygenic traits) and other associations may be large.

31 Many groups have joined efforts to create large consortia that assemble results from multiple  
32 GWAS, providing aggregated sample sizes that are now in excess of a million individuals (Linnér et al.  
33 2019; Lee et al. 2018). Meta-analysis of consortia datasets improves the power necessary to detect  
34 many genotype-phenotype associations. However, where population structure exists in a dataset  
35 but is insufficiently controlled for, it can lead to spurious or inflated genotype-phenotype  
36 associations (Lawson et al. 2020; Peterson et al. 2017). Even within the UK, considering only white  
37 people of European ancestry, migration and socio-economic position correlate with ancestry  
38 (Abdellaoui et al. 2019; Haworth et al. 2019).

39 Recently it has become apparent that GWAS results based on large scale meta-analysis have been at  
40 least partially biased due to inadequate correction for confounding by population stratification. The  
41 Genetic Investigation of ANthropometric Traits (GIANT Consortium 2018) meta-analysis of height  
42 and BMI (Wood et al. 2014; Locke et al. 2015) has led to ambiguous conclusions regarding selection  
43 on height (Yengo et al. 2018) with Genetic Scores being of particularly discussion vulnerable to this  
44 confounding (Berg et al. 2019; Sohail et al. 2019). Similar issues have been reported for Educational  
45 Attainment (Abdellaoui et al. 2019; Haworth et al. 2019), as well as and diseases including Type 2  
46 diabetes and coronary heart disease (Reisberg et al. 2017).

47 Latent structure and population stratification are addressed during the discovery of associated  
48 genetic variants by correcting for Principal Components (PCs) of the genetic variation (Price et al.  
49 2006). Historically, only a few PCs were used, increasing with sample size and time from two  
50 (Wellcome Trust Case Control Consortium 2007), five (Warrington et al. 2015) and ten (Okbay et al.  
51 2016) to 40 - the default provided by UK Biobank (Bycroft et al. 2018) – to 100 or more (Abdellaoui  
52 et al. 2019). Yet even 100 PCs are insufficient (Lawson et al. 2020) as important structures may  
53 explain less variation than noise and hence remain uncorrected, which can lead to uneven correction  
54 and bias in meta-analysis.

55 We propose a simple solution. Correction can be improved and standardized using a large external  
56 reference dataset to define “all human genetic variation”, against which local variation within a  
57 single study can be compared. Thus, whilst population stratification might act as a source of  
58 covariance between genotypes and phenotypes, this can be corrected for. We demonstrate that  
59 meta-analyses corrected for population stratification using a large external reference dataset  
60 (“global” ancestry correction) performs better than meta-analyses corrected for population  
61 stratification using the same dataset (“local” ancestry) in the UK Biobank.

62 There are alternative methodologies for stratification correction that go beyond PC correction.  
63 Linear Mixed Models (LMMs) have gained popularity since their introduction in genetics (Yu et al.  
64 2006) through easy-to-use software such as GCTA (Yang et al. 2011). LMMs are a “gold-standard” for  
65 GWAS because instead of correcting for only the top few variance components, they correct in  
66 principle for the entire Genetic Relatedness Matrix (GRM) comparing all pairs of individuals. This  
67 allows familial structure to be corrected in the same framework as ancestry. However, whilst  
68 pairwise relationships in the GRM are measured in the data, correlations between them are still  
69 estimated with noise and hence correction performance improves with sample size. We use Bolt-  
70 LMM (Loh et al. 2015) throughout and find that correction for external PCs complements, and is not  
71 replaced by, the use of LMMs.

72 We investigate the relationship between latent genetic structure and phenotypes, i.e. population  
73 stratification, in the UK Biobank. We demonstrate that proper correction for stratification has  
74 implications in the Avon Longitudinal Study of Parents and Children (ALSPAC) (Boyd et al. 2013;  
75 Fraser et al. 2013) in Bristol, UK, especially were the results are to be considered as part of a meta-  
76 analysis. These findings provide evidence that similar correction will lead to changes in findings for  
77 large-scale meta-analysis.

78 Software and appropriate reference data are provided (see Code Availability) to allow others to  
79 easily apply this to their own data.

## 80 Results

### 81 Identification of population structure required for correction

82 Successful identification and prioritization of disease-associated causal variants relies on  
83 understanding the distribution of genetic variants within and between populations. However, the  
84 extent to which ancestry can impact variant frequencies is not always clear. Accurate understanding  
85 and use of methods of correcting for ancestry such as PCs is critical.

86 We are interested in constructing and improving ancestry inference for all studies. To this aim we  
87 constructed 200 PCs (see Materials and Methods) following the sample and SNP selection and PC  
88 computation methodology of (Bycroft et al. 2018). Critically, PC loadings and eigenvalues are made  
89 available, allowing projection of external datasets into this ancestry measure, which we refer to as  
90 “global” ancestry/PCs. This contrasts to “local” ancestry and PCs, constructed using PC analysis  
91 within a single dataset.

92 The global moniker implies usefulness outside of the UK. The UK Biobank remains one of the largest  
93 easily accessed resources for worldwide variation, including (with some arbitrary choices of  
94 definition) over 6k Sub-Saharan Africans, 2k East Asians, and 7k South Asians. Naturally, a larger  
95 reference would identify further local structure. Similar to a recent study (Privé et al. 2020), we  
96 found evidence (Supplementary Figure S1) that Linkage Disequilibrium (LD) is important after the  
97 first 18 PCs, that ancestry associations reduce after 40 PCs, and that some population structure is  
98 associated with further PCs (Materials and Methods).

### 99 Population Structure in the UK Biobank

100 We restricted our stratification analyses to 331,890 UK Biobank participants of UK ancestry  
101 excluding Northern Ireland, and ~12M SNPs after quality-control filtering and LD pruning (see  
102 Materials and Methods). For illustration purposes, we clustered individuals using k-means (see  
103 Materials and Methods) into 5 clusters (Figure 1a). The largest cluster represented southern and  
104 eastern England, with northern England, Scotland, North Wales, and South Wales each being  
105 represented (Galinsky et al. 2016) . We are not attempting to infer actual ancestry from these PCs.

106 PCs are ordered by the total variation explained in the data. Major variation directions are  
107 associated with deep historical splits between populations such as African vs Eurasians (PC1-2),  
108 Europeans vs East Asians (PC1-3), Central Asia (PC3-4), and Europe (PCs 5,8). This contrasts regional  
109 variation within the UK for which the main PCs are 5 and 9 describing variation between English,  
110 Scottish and Welsh ancestry, as well as PCs 11 and 14 which further separate structure within Wales  
111 and England. This is strongly structured by study centre, which captures current living location  
112 (Figure 1b). These and other PCs (Supplementary Figure S2-3) correspond to known historical and  
113 geographical areas (Leslie et al. 2015).

114 To assess how much of this variation is captured by local PCs, we performed PC projection, i.e. a  
115 regression analysis for each global PC using all local PCs as predictors (see Materials and Methods).  
116 Local PCs capture global variation with varying veracity (Figure 1c). The predictability of global PCs  
117 varies by study centre according to which populations are poorly represented in them. PC5 is best  
118 explained in the West and describes Welsh vs English ancestry. PC9 describes South Wales ancestry;  
119 PC11 describes northern England ancestry; PC14 describes Scottish ancestry; whilst PC16 describes  
120 North Welsh ancestry. Worldwide ancestry PCs are homogeneous within the UK and therefore  
121 cannot be explained (PC1-3,6-8,13,17). Local PCs for all 22 study centres fail to explain some UK  
122 ancestry, and the inverse prediction of explaining local PCs using Global PCs shows that the local  
123 analyses typically contain only 2-4 ancestry related PCs (Figure 1d).

124 This observed population structure within the UK provides a source of covariance between  
125 genotypes and phenotypes that can bias epidemiological inference from genetic data. The following  
126 sections establish consequences of unexplained covariance for understanding complex disease.

### 127 [Stratification correction using global vs local PCs in UK Biobank](#)

128 The most straightforward measure of stratification is of the total variation in phenotypes explained  
129 by genetic PCs, without attributing this to individual SNPs. Both educational attainment (EA) and  
130 Body Mass Index (BMI) vary by region (Supplementary Figure S4) and show large systematic  
131 differences between local ancestry and global ancestry correction (Figure 2). Several study centres  
132 explain dramatically less variation with local PCs than global, for example for EA in Croydon (0.6%  
133 local vs 3.2% global) and Hounslow (0.8% local vs 3% global). Figure 1c-d explains this as a failure to  
134 identify components corresponding to Scottish, Welsh and other ancestries that are individually rare  
135 but nevertheless important when considered together. Conversely others, especially centres with  
136 small sample sizes such as Wrexham and Swansea, explain more variation in local than global  
137 ancestry.

138 We tested 24 disease statuses for the amount of variance explained by Local or Global PC correction,  
139 and found that Psoriasis, Hyperthyroidism, and Hypothyroidism were all significant different (Figure  
140 S5) and Multiple Sclerosis and Asthma are implicated though not significant after correcting for  
141 multiple testing.

142 Our analyses demonstrate two competing effects. Firstly, local PCs in small studies “overfit”, as they  
143 are able to explain much of the variance present regardless of whether it describes real ancestry or  
144 noise. This is why the number of PCs corrected for is often thresholded using a noise-level  
145 approximation (Lawson and Falush 2012) and justifies the small number of PCs used in early GWAS.  
146 Secondly, some ancestry components will not be recovered in a small dataset due to lack of  
147 statistical power. Mathematically, PC analysis displays a transition as sample size decreases, in which  
148 a particular population structure is identified when enough variation exists for it, and rather abruptly  
149 becomes indistinguishable from noise (McVean 2009). Importantly, local PCs perform worse not  
150 solely in small studies, but in larger but genetically more homogenous populations of the South-East  
151 of England. It is rare shared variation, regardless of the size of the study, that local PCs fail to identify  
152 and hence correct for.

### 153 [Local vs global correction for individual GWAS Effect sizes in UK Biobank](#)

154 Meta-analysis is a statistical tool for combining results from coherent studies on different samples. A  
155 fundamental principle in GWAS meta-analysis is that all studies included examined the same  
156 hypothesis, had similar study design and analyzed study-level SNPs in a near-identical way (Zeggini  
157 and Ioannidis 2009; Bush and Moore 2012; Evangelou and Ioannidis 2013), similar imputation (Li et  
158 al. 2009), quality control, large-scale ancestry (Peterson et al. 2017) and of course, population  
159 stratification correction. Meta-analysis is individually important and offers a chance to examine  
160 stratification correction entirely within the (supposedly) homogeneous UK Biobank cohort.

161 For EA and BMI we estimated effect sizes when performing meta-analysis with global and local PC  
162 correction in the UK Biobank. Whilst individually, most SNP effect changes are not statistically  
163 significant, three issues arise (Figure 3). Firstly, estimates are systematically larger in magnitude  
164 when correcting with local rather than global ancestry. Secondly, some subsets of SNPs respond in a  
165 systematically different way (Supplementary Figure S6), leading to “clusters” of SNPs that are under,  
166 or over, corrected using local ancestry alone. Finally, smaller effects with the least statistical support  
167 are larger with local correction; by 2% in EA, 0.6% for BMI for genome-wide significant SNPs  
168 (determined by regressing local estimates on global; Supplementary Figure S6).

169 These results are consistent with the proportion of variance in different phenotypes (e.g. education  
170 attainment and BMI) being larger when corrected for global PCs than local PCs (Supplementary  
171 Figure S7). The magnitude of the difference varies between phenotypes, and depends on the causal  
172 model i.e. the relationships between phenotype, genotype, ancestry, and geography (Lawson et al.  
173 2019).

#### 174 [Reference PCs can be used to identify structure: a case study in ALSPAC](#)

175 To test our hypothesis that uncorrected population structure may lead to misleading inference, we  
176 examined the ALSPAC cohort. Local variation is lost when effective sample size for a particular  
177 ancestry reduces beyond a threshold. We compare two studies in Bristol, the UK Biobank (N=27,503)  
178 and ALSPAC (N=7,927 mothers in our analysis). When constructing global ancestry using the entire  
179 UK Biobank variation, the two datasets have very similar genetic variation profiles across all PCs  
180 (Figure 4), including the main structures such as varying Scottish/English ancestry proportions.  
181 However, the datasets differ when projecting local ancestry PCs constructed from within each  
182 dataset into global ancestry (see Materials and Methods). Local PCs of the larger UK Biobank Bristol  
183 centre dataset partially recover most of the UK variation, whilst PCs of the smaller ALSPAC dataset  
184 recover very little. This would lead to systematic under-correction if replicated across a meta-  
185 analysis.

186 But does this matter for understanding phenotypes? To answer this question, we examined several  
187 phenotypes that have been studied with well-powered GWAS, including BMI, Educational  
188 attainment, IQ and C-reactive protein (CRP). We estimated the effect size in ALSPAC for both the  
189 study mothers and study children for SNPs identified by previous studies (see Materials and  
190 Methods) correcting either for local or global PCs.

191 Summarizing the total variance explained for phenotypes (Figure 5a) we find that the global PCs  
192 explain more variation in EA, IQ and BMI, but not CRP. This is most dramatic for mothers' EA for  
193 which 7% vs 1% (global vs local) of variation is explained, matching previous estimates using  
194 haplotype information (Lawson et al. 2012) to quantify population structure in ALSPAC (Haworth et  
195 al. 2019; Lawson et al. 2020).

196 As ALSPAC is a relatively small cohort, the uncertainty involved in SNP effect estimation dominates  
197 the results. However, we found that the more robust estimates (higher Z-scores) changed  
198 systematically between correction models (Figure 5b, Supplementary Figure S8). Intriguingly, the  
199 direction is not the same for all phenotypes; local correction results in relatively larger estimates (i.e.  
200 under-correction) for EA, whilst it results in smaller estimates for BMI, which could imply subtle  
201 relatedness or improved power from correcting for ancestry.

202 Constructing a Genetic Score using this procedure leads to a similar picture, with systematic biases in  
203 prediction (Figure 5c, Supplementary Figure S9). Whilst there is statistical power to detect some  
204 differences in the scaled scores (e.g. in EA and CRP) these are unlikely to be practically significant  
205 changes. We therefore view the ordering of individuals to have been robust in this example.  
206 However, the raw scores are strongly skewed, again with biases in both directions, and further, the  
207 bias direction appears unrelated to whether SNPs were individually over or under predicted.

#### 208 [Providing an appropriate set of ancestry covariates](#)

209 The primary barrier to using the UK Biobank PCs is a lack of access to a) SNP loadings, and b)  
210 reference information to scale SNPs and perform QC carefully. We provide the key 18 ancestry PCs  
211 plus SNP information in an R package and script (see Code Availability) which allows trivial access to



212 for all datasets in plink bim/bed/fam format of any size (e.g. runs on all 500k UK Biobank individuals  
213 in 6 hours). We further provide up to 200 UK Biobank PCs.

214 Users with access to UK Biobank data should consider the *bigsnp* R package (Privé et al. 2020) which  
215 allows translation of any dataset into UK Biobank PCs with careful quality control assured due to  
216 comparison with the original raw data. Advanced users who do their own quality control and  
217 imputation may wish to directly apply the *flashpca* software (Abraham, Qiu, and Inouye 2017) to our  
218 provided reference data. Our package provides strand and build checks, automatically merges data  
219 coded with different minor alleles, and accounts for a moderate amount of non-overlapping SNPs.

220 Above, our UK Biobank results used BoltLMM (Loh et al. 2015). We confirm that these results are not  
221 meaningfully different to what we would have seen using linear regression correcting for PCs with  
222 PLINK (Supplementary Figure S10). The ALSPAC results also used PLINK. Therefore the effects  
223 describe are confirmed to apply to both linear regression and linear mixed models using the  
224 BoltLMM approximation.

## 225 Discussion

226 Population stratification in association studies has received much attention. However, it has typically  
227 been considered as a problem of unintended correlations within the dataset, leading to correction in  
228 the form of a within-sample analysis (using PCA or other approaches). We provide evidence that this  
229 framing is insufficient. Whilst it is indeed unintended correlations that we wish to correct for,  
230 population structure is not always detectible from the dataset being studied. This hard-to-quantify  
231 population structure can be structurally related to phenotypes.

232 We demonstrated that within the UK Biobank's individual study centres with samples of tens of  
233 thousands, as well as in the independent ALSPAC cohort, correcting for population stratification with  
234 a high-quality, external measure of population structure is necessary. Population structure exists at  
235 the within-city level and it is not correctly quantified within geographically clustered datasets. We  
236 found considerable residual correlation with phenotypes and identified that the SNP-level estimates  
237 were systematically biased. This resulted in appreciable error at the genome-wide level for the  
238 construction of Genetic Scores.

239 We identified that, were the UK Biobank to have been analysed as independent study centres  
240 subject to meta-analysis, then Educational Attainment, BMI, Psoriasis, Hyperthyroidism and  
241 Hypothyroidism would all have led to biased inference. This is likely to be the tip of the iceberg in  
242 meta-analyses, since the UK is a rather homogeneous population and the power in rare diseases is  
243 low.

244 Because global PCs are unarguably a better measure of population structure, it is tempting to  
245 interpret the effect size for the global PC correction as "more correct" than that for the local PC  
246 correction, and therefore the difference as a bias with the traditional approach. However, it is not  
247 that simple. We found little consistency in the direction of the bias; for example, EA for ALSPAC  
248 children appears to be "undercorrected" by local PCs, whereas the mothers EA appeared  
249 "overcorrected". The reality is that confounding is caused by many sources, and shared ancestry is  
250 just one. Here we suspect that cryptic relatedness may exist, which is captured only by the local PCs.

251 The informed reader may find these results self-evident. However, the evidence that we provide of  
252 the importance and ease of improved stratification correction has clear implications. Future meta-  
253 analyses and association studies should adopt a new protocol for quantifying population  
254 stratification. Further, every analysis of small to medium sized cohorts whose association outputs

255 remain of value should be re-considered. Large meta-analyses are particularly valuable and yet  
256 vulnerable to the biases identified here. Similarly, phenotypes with a non-trivial social or  
257 environmental component (Morris et al. 2020) are likely to be influenced by this or other hidden  
258 structural biases.

259 The new protocol should continue to adjust for relatedness within the cohort, but it must also add  
260 the confounding covariates of ancestry as quantified by a large and hence statistically powerful  
261 external resource. We provide such “genetic measures” for the UK Biobank reference in the form of  
262 PC loadings that can project any individual into this worldwide quantification of genetic variation.

263 Yet for non-UK individuals, even in the UK Biobank, this may be insufficient. There is no reason that  
264 institutions with access to large limited-access databases could not make and share independent PC  
265 loadings, for every region of the world, that smaller association studies with less power individually  
266 can apply. Although this is a partial solution because a nuanced quantification of ancestry is not  
267 linear, these sharable PCs will improve stratification correction with trivial cost, so the genetics  
268 community can and should implement this immediately.

## 269 Data and Code availability

270 [github.com/danjlawson/pcapred](https://github.com/danjlawson/pcapred): R package for projecting into UK Biobank variation.

271 [github.com/danjlawson/pcapred-script](https://github.com/danjlawson/pcapred-script): Script for non-R users to perform command line projection.

272 [github.com/danjlawson/pcapred-data](https://github.com/danjlawson/pcapred-data): 200 ancestry PCs for UK Biobank.

273 ALSPAC ([www.bristol.ac.uk/alspac/researchers/access/](http://www.bristol.ac.uk/alspac/researchers/access/)) and UK Biobank data  
274 ([www.ukbiobank.ac.uk/principles-of-access/](http://www.ukbiobank.ac.uk/principles-of-access/)) are both accessible under their respective data use  
275 policies.

## 276 Materials and Methods

### 277 Cohorts

#### 278 UK Biobank

279 The UK Biobank is a population-based health research resource consisting of approximately 500,000  
280 people, aged between 38 years and 73 years, who were recruited between the years 2006 and 2010  
281 from across the UK (Sudlow et al. 2015), particularly focused on identifying determinants of human  
282 diseases in middle-aged and older individuals, participants provided a range of information (such as  
283 demographics, health status, lifestyle measures, cognitive testing, personality self-report, and physical  
284 and mental health measures) via questionnaires and interviews; anthropometric measures, BP  
285 readings and samples of blood, urine and saliva were also taken (data available at  
286 [www.ukbiobank.ac.uk](http://www.ukbiobank.ac.uk)). A full description of the study design, participants and quality control (QC)  
287 methods have been described in detail previously (Bycroft et al. 2018). UK Biobank received ethical  
288 approval from the Research Ethics Committee (REC reference for UK Biobank is 11/NW/0382). Access  
289 was under Application ID 21829.

#### 290 ALSPAC

291 Pregnant women resident in Avon, UK with expected dates of delivery 1st April 1991 to 31st December  
292 1992 were invited to take part in the study. The initial number of pregnancies enrolled is 14,541. Of  
293 these initial pregnancies, there was a total of 14,676 fetuses, resulting in 14,062 live births and  
294 13,988 children who were alive at 1 year of age. When the oldest children were approximately 7 years  
295 of age, an attempt was made to bolster the initial sample with eligible cases who had failed to join the

296 study originally. The total sample size for analyses using any data collected after the age of seven is  
297 therefore 15,454 pregnancies, resulting in 15,589 fetuses. Of these 14,901 were alive at 1 year of  
298 age. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the  
299 Local Research Ethics Committees. Consent for biological samples has been collected in accordance  
300 with the Human Tissue Act (2004). Informed consent for the use of data collected via questionnaires  
301 and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and  
302 Law Committee at the time. For further details of the cohort please see (Boyd et al. 2013; Fraser et al.  
303 2013). Please note that the study website contains details of all the data that is available through a  
304 fully searchable data dictionary and variable search tool  
305 (<http://www.bristol.ac.uk/alspac/researchers/our-data/>).

## 306 Genotyping, imputation and quality control

### 307 PCA Analysis

308 PCA analysis of the UK biobank was performed with *flashPCA* (Abraham, Qiu, and Inouye 2017) after  
309 following the procedure described in (Bycroft et al. 2018) to subset SNPs (147604 retained) and  
310 individuals (406758 retained). *FlashPCA* reports standardized Eigenvectors, unlike *FastPCA* (Galinsky  
311 et al. 2016) as used and reported by UK Biobank which scales Eigenvectors using the Eigenvalues. For  
312 stratification correction the distinction is not important, and our tool *pcapred* can translate between  
313 the two.

### 314 UK Biobank

315 The full data release contains the cohort of successfully genotyped samples (n=488,377). 49,979  
316 individuals were genotyped using the UK BiLEVE array and 438,398 using the UK Biobank axiom array.  
317 Pre-imputation QC, phasing and imputation are described elsewhere (Bycroft et al. 2018). In brief,  
318 prior to phasing, multiallelic SNPs or those with MAF  $\leq 1\%$  were removed. Phasing of genotype data  
319 was performed using a modified version of the SHAPEIT2 algorithm (O'Connell et al. 2016). Genotype  
320 imputation to a reference set combining the UK10K haplotype and HRC reference panels 8was  
321 performed using IMPUTE2 algorithms (Howie, Marchini, and Stephens 2011). The analyses presented  
322 here were restricted to autosomal variants within the HRC site list using a graded filtering with varying  
323 imputation quality for different allele frequency ranges. Therefore, rarer genetic variants are required  
324 to have a higher imputation INFO score (Info>0.3 for MAF >3%; Info>0.6 for MAF 1-3%; Info>0.8 for  
325 MAF 0.5-1%; Info>0.9 for MAF 0.1-0.5%) with MAF and Info scores having been recalculated on an in-  
326 house derived 'European' subset (Mitchell et al. 2019).

327 Individuals with sex-mismatch (derived by comparing genetic sex and reported sex) or individuals with  
328 sex-chromosome aneuploidy were excluded from the analysis (n=814).

329 We restricted the sample to individuals of 'european' ancestry as defined by an in-house k-means  
330 cluster analysis performed using the first 4 principal components provided by UK Biobank in the  
331 statistical software environment R. The current analysis includes the largest cluster from this analysis  
332 (n=464,708) (Mitchell et al. 2019).

### 333 ALSPAC

334 DNA of the ALSPAC children was extracted from blood, cell line and mouthwash samples, then  
335 genotyped using references panels and subjected to standard quality control approaches. ALSPAC  
336 children were genotyped using the Illumina HumanHap550 quad chip genotyping platforms by  
337 23andme subcontracting the Wellcome Trust Sanger Institute, Cambridge, UK and the Laboratory  
338 Corporation of America, Burlington, NC, US. The resulting raw genome-wide data were subjected to  
339 standard quality control methods. Individuals were excluded on the basis of gender mismatches;



340 minimal or excessive heterozygosity; disproportionate levels of individual missingness (>3%) and  
341 insufficient sample replication (< 0.8). Population stratification was assessed by multidimensional  
342 scaling analysis and compared with Hapmap II (release 22) European descent (CEU), Han Chinese,  
343 Japanese and Yoruba reference populations; all individuals with non-European ancestry were  
344 removed. SNPs with a minor allele frequency of < 1%, a call rate of < 95% or evidence for violations of  
345 Hardy-Weinberg equilibrium ( $P < 5 \times 10^{-7}$ ) were removed. Cryptic relatedness was measured as  
346 proportion of identity by descent (IBD) > 0.1. Related subjects that passed all other quality control  
347 thresholds were retained during subsequent phasing and imputation. 9,115 participants and 500,527  
348 SNPs passed these quality control filters. ALSPAC mothers were genotyped using the Illumina  
349 human660W-quad array at Centre National de Génotypage (CNG) and genotypes were called with  
350 Illumina GenomeStudio. PLINK (v1.07) was used to carry out quality control measures on an initial set  
351 of 10,015 subjects and 557,124 directly genotyped SNPs. SNPs were removed if they displayed more  
352 than 5% missingness or a Hardy-Weinberg equilibrium P value of less than  $1.0 \times 10^{-6}$ . Additionally, SNPs  
353 with a minor allele frequency of less than 1% were removed. Samples were excluded if they displayed  
354 more than 5% missingness, had indeterminate X chromosome heterozygosity or extreme autosomal  
355 heterozygosity. Samples showing evidence of population stratification were identified by  
356 multidimensional scaling of genome-wide identity by state pairwise distances using the four HapMap  
357 populations as a reference, and then excluded. Cryptic relatedness was assessed using an IBD estimate  
358 of more than 0.125 which is expected to correspond to roughly 12.5% alleles shared IBD or a  
359 relatedness at the first cousin level. Related subjects that passed all other quality control thresholds  
360 were retained during subsequent phasing and imputation. 9,048 subjects and 526,688 SNPs passed  
361 these quality control filters.

362 We combined 477,482 SNP genotypes in common between the sample of mothers and sample of  
363 children. We removed SNPs with genotype missingness above 1% due to poor quality (11,396 SNPs  
364 removed) and removed a further 321 subjects due to potential ID mismatches. This resulted in a  
365 dataset of 17,842 subjects containing 6,305 duos and 465,740 SNPs (112 were removed during liftover  
366 and 234 were out of HWE after combination). We estimated haplotypes using ShapIT (v2.r644) which  
367 utilises relatedness during phasing. The phased haplotypes were then imputed to the Haplotype  
368 Reference Consortium (HRCr1.1, 2016) panel of approximately 31,000 phased whole genomes. The  
369 HRC panel was phased using ShapIT v2, and the imputation was performed using the Michigan  
370 imputation server. This gave 8,237 eligible children and 8,196 eligible mothers with available genotype  
371 data after exclusion of related subjects using cryptic relatedness measures described previously.  
372 Principal components were generated by extracting unrelated individuals (IBS < 0.05) and  
373 independent SNPs with long range LD regions removed, and then calculating using the `--pca`  
374 command in plink1.90.

### 375 [Association analysis: statistical methods](#)

376 Genome-wide association analysis (GWAS) was conducted using linear mixed model (LMM)  
377 association method as implemented in BOLT-LMM (v2.3) (Loh et al. 2015). To model population  
378 structure in the sample we used 143,006 directly genotyped SNPs, obtained after filtering on MAF >  
379 0.01; genotyping rate > 0.015; Hardy-Weinberg equilibrium p-value < 0.0001 and LD pruning to an  $r^2$   
380 threshold of 0.1 using PLINKv2.00. Genotype array and sex were adjusted for in the model. BOLT-LMM  
381 association statistics are on the linear scale. As such, test statistics (betas and their corresponding  
382 standard errors) were transformed to log odds ratios and their corresponding 95% confidence  
383 intervals on the liability scale using a Taylor transformation expansion series (Loh et al. 2015).

## 384 Meta-analysis

385 Meta-analysis for variance explained was conducted using *rma* from the “metafor” package for R  
386 (Viechtbauer 2010) using the normal distribution approximation. P-values for the difference in  $R^2$  were  
387 calculated by computing the difference in the estimates, and the variance of the difference (the sum  
388 of the individual variances) and using the null that the  $R^2=0$  again using *rma*. For binary traits, only  
389 study centres with at least 20 cases were considered. We also implemented a bootstrap procedure  
390 that did not make the normal distribution approximation, in which study centres were resampled 500  
391 times with replacement. However, the results were not qualitatively different (not shown).

## 392 Polygenic scoring

393 Genetic scores were created in the ALPAC cohort using PLINK (Purcell et al. 2007) based upon the list  
394 of SNPs identified to associate with educational attainment (Lee et al. 2018), BMI (Yengo et al. 2018),  
395 IQ (Lee et al. 2018) and CRP (Ligthart et al. 2018) in previous GWAS. All SNPs were weighted by their  
396 effect size in the replication cohort of the GWAS, and these sizes were summed using allelic scoring.  
397 The genetic scores were generated using GWAS results which had removed the ALSPAC cohort where  
398 included in the original GWAS, and therefore the scores are not perfectly comparable to those  
399 reported in the published meta-analysis. Where the lead SNPs from GWAS were not available in  
400 ALSPAC, we instead used the SNPs in highest linkage disequilibrium. Genetic score analysis in ALSPAC  
401 was run on age and sex standardised phenotypes controlling for either local PCs (the first 20 principal  
402 components of ancestry as identified within the ALSPAC cohort) or global PCs (the first 20 principal  
403 components of ancestry constructed from UK Biobank loadings).

## 404 ALSPAC phenotypes

405 For ALSPAC mothers, years of education was determined by recoding highest level of education  
406 reported during pregnancy. Response were coded as basic formal education (7 years), certificate of  
407 secondary education (10 years), O-levels and vocational qualifications (11 years), A-level (13 years),  
408 and degree (16 years). Mother’s BMI was measured during the ‘Focus on Mothers 1’ direct assessment  
409 when the study offspring were aged 17 (mother ages 34-63).

410 For ALSPAC children, education was measured using average fine graded point scores in age 16  
411 educational examinations, which represents final compulsory schooling examinations. Scores were  
412 obtained through data linkage to the UK National Pupil Database (NPD), which represents the most  
413 accurate record of individual educational achievement available in the UK. Intelligence was measured  
414 during the direct assessment at age eight using the short form Wechsler Intelligence Scale for Children  
415 (WISC) (Wechsler 1992) from verbal, performance, and digit span tests and administered by members  
416 of the ALSPAC psychology team overseen by an expert in psychometric testing. Raw scores were  
417 recalculated to be comparable to those that would have been obtained had the full test been  
418 administered and then age-scaled to give a total overall score combined from the performance and  
419 verbal subscales. BMI was measured during the direct assessments at ages 7, 8, 9, 10 and 11. In order  
420 to increase sample size, where BMI data were not available at age 7 we used BMI measured at the  
421 earliest available subsequent measurement. C-reactive protein (CRP) was measured from non-fasting  
422 blood assays taken during direct assessment when the offspring were aged 9.

## 423 Detecting bias in scores and SNP effects

424 To assess statistical power, we work with z-scores, i.e.  $z_i = \hat{\beta}_i / \hat{\sigma}_i$ , where  $\hat{\beta}_i$  is the estimate of the  
425 effect of SNP  $i$  and  $\hat{\sigma}_i$  is the estimate of the standard deviation of this estimate. To compare the  
426 global (g) and local (l) effects we consider the mean estimate  $\bar{z}_i = (z_{g,i} + z_{l,i})/2$  and difference  $\partial_i =$   
427  $(z_{g,i} - z_{l,i})$  for each SNP  $i$ . To prevent the large number of barely-significant estimates from  
428 dominating the signal, we assign a weight to each SNP  $w_i = 1/\rho_i$  where  $\rho_i$  is the density estimate

429 taken from a 5 nearest-neighbour estimate using “knnDE” from the R Package “TDA”. We then  
430 perform robust regression for  $\partial \sim z$  and report the regression estimate and confidence interval. We  
431 further checked that our conclusions are not impacted by these choices by performing regular  
432 unweighted regression for  $\partial \sim z$ .

### 433 UK Biobank trait definition

434 Years of education was determined by recoding highest level of education reported in a questionnaire.  
435 Response were coded as basic formal education (7 years), O-levels/GCSEs/CSEs or equivalent (10  
436 years), A-level/AS levels or equivalent (13 years), NVQ or HND or HNC or equivalent (19 years) and  
437 College/University degree (20 years). We also binary studied educational attainment (EA), which is  
438 measured as 1 for people who have obtained a College or University degree.

439 Height and weight were measured during the participants’ baseline visit to a UK Biobank assessment  
440 center.

441 Heel bone mineral density (eBMD) was estimated based on an ultrasound measurement of the  
442 calcaneus by UK Biobank. The T-score is the number of standard deviations for bone mineral density  
443 relative to the mean. Consistent with the criteria established by Kemp et al., individuals were  
444 excluded that exceeded the following thresholds for eBMD: males,  $\leq 0.18$  or  $\geq 1.06$  g/cm<sup>2</sup>; females  
445  $\leq 0.12$  or  $\geq 1.025$  g/cm<sup>2</sup>.

446 Other traits were self-reported at the verbal interview and coded as yes/no. If the participant was  
447 uncertain of the type of illness they had had, then they described it to the interviewer (a trained  
448 nurse) who attempted to place it within the coding tree. If the illness could not be located in the  
449 coding tree then the interviewer entered a free-text description of it. These free-text descriptions  
450 were subsequently examined by a doctor and, where possible, matched to entries in the coding tree.

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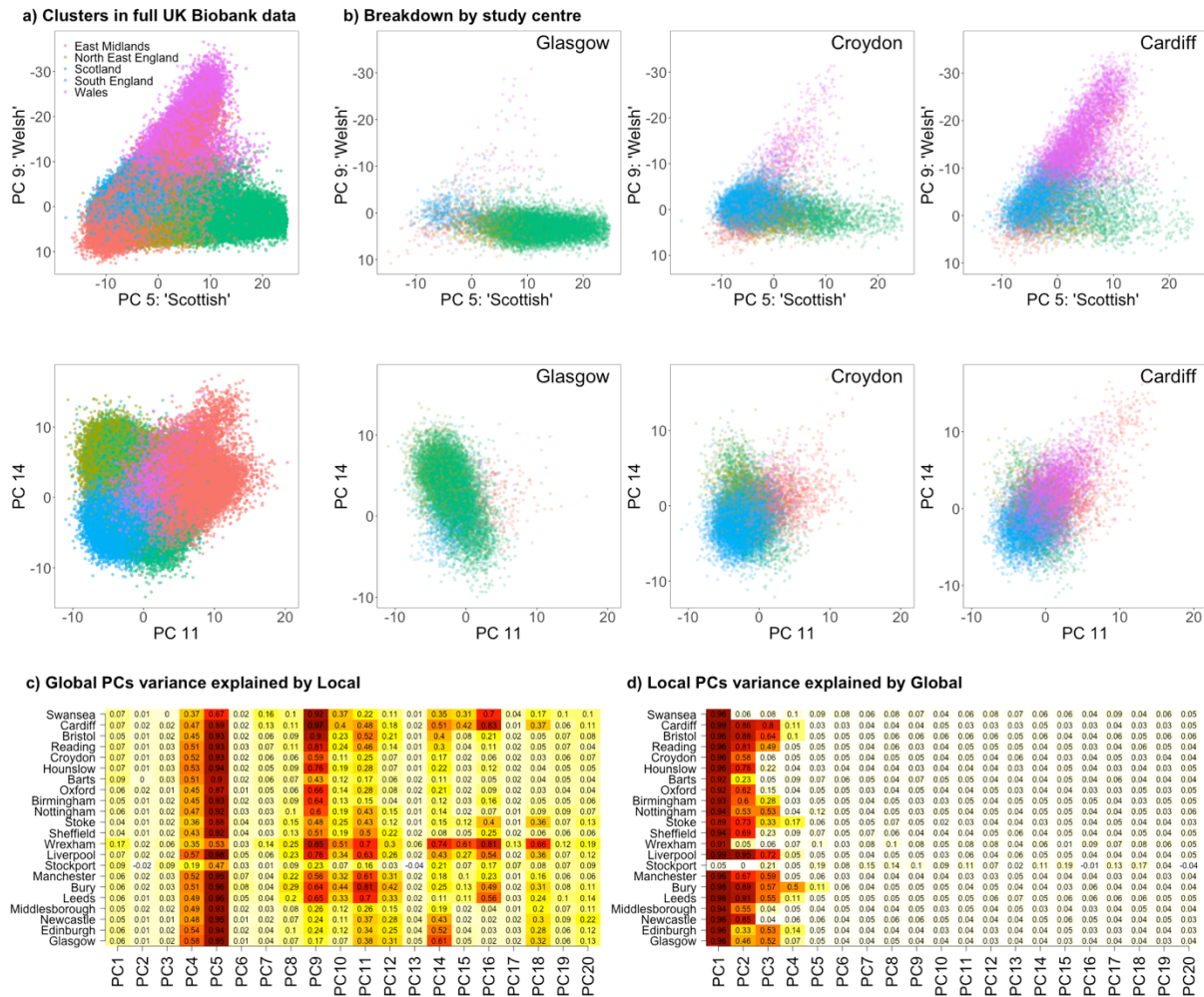
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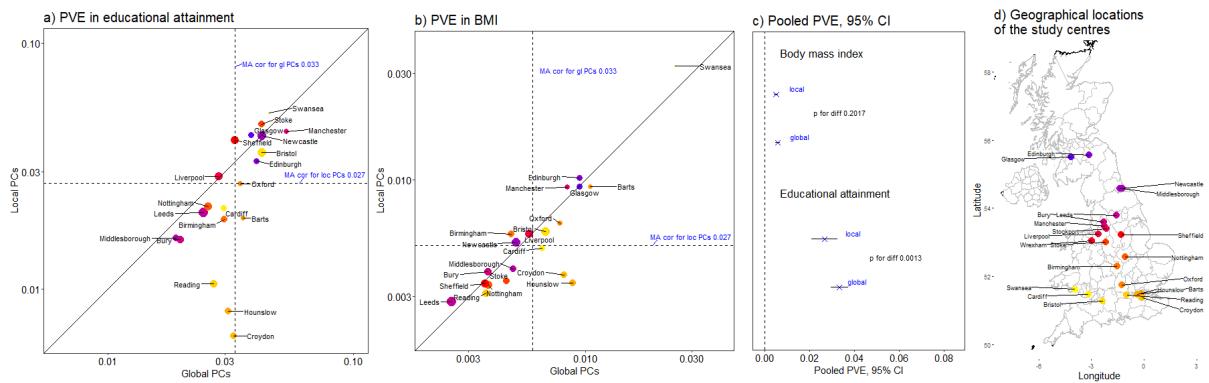
622 Figure 1. UK Biobank PCs by study centre

623 Global (i.e. inferred in the full UK Biobank) genetic ancestry PCs (Principal Components) is  
 624 incompletely captured by local ancestry. a) The Global (whole biobank) PC analysis reveals  
 625 British ancestry primarily in PCs 5,9,11 and 14 (see Supplementary Figure S2). b) Retaining  
 626 PCs only for one geographical study centre at a time shows that many ancestries are under-  
 627 sampled. c) Conducting a PC analysis within a single study centre, and trying to recover the  
 628 PCs (see Methods), leads to low variance explained ( $R^2$ ) for many PCs. d) Predicting in  
 629 reverse, only the first 2-5 PCs of a local analysis capture ancestry, with the remaining PCs  
 630 being non-significant and are shown in pale with a white border (see Methods).  
 631



632  
633

634 Figure 2. Stratification correction bias seen in Proportion of Variance Explained (PVE)  
635 Meta-analysis of UK Biobank study centres demonstrates stratification problems. a) Proportion of  
636 Variance Explained (PVE) in Educational attainment corrected for 40 global vs 40 local PCs, split by  
637 study centre. The point size indicate sample size per study centre, and colours show geography (d).  
638 b) Proportion of Variance Explained in BMI (Body Mass Index). c) Pooled PVE and 95% Confidence  
639 Intervals, with p-values for a paired t-test for a difference in mean. d) The Geographical locations  
640 of the study centres explaining the colour gradient: from Scotland/North (blue) to Midlands (red), via  
641 South-East (orange) to Wales/South-West (yellow) .

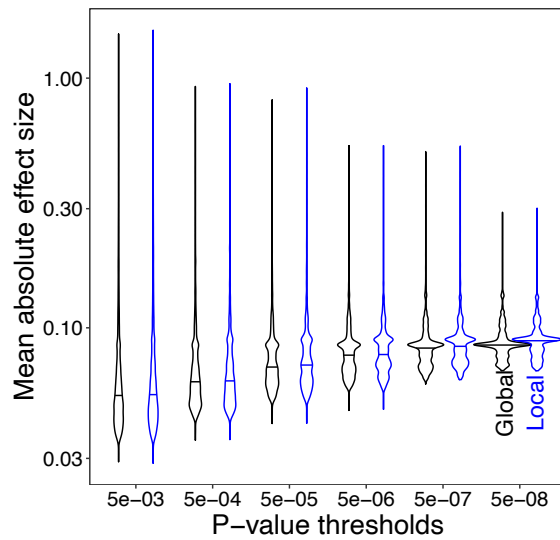


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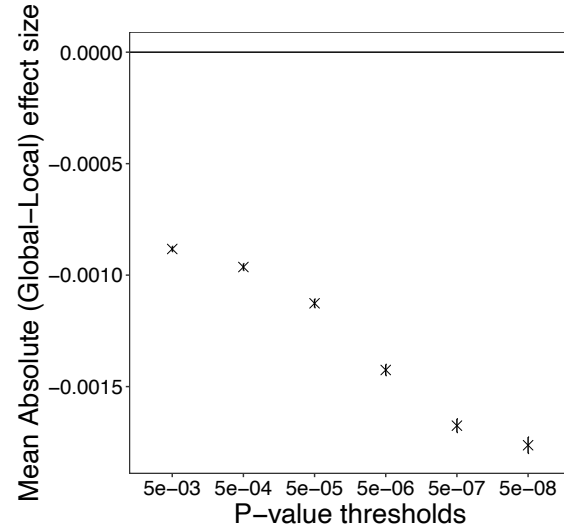
643

644 **Figure 3. Stratification of SNP effect size bias in UK Biobank (education years)**  
645 Stratification correction changes UK Biobank effect size estimates and the magnitude of the change  
646 varies by significance threshold. a) The mean absolute effect size for educational years and its  
647 median value as a function of p-value threshold, for Global or Local PC corrected meta-analysis. b)  
648 Mean absolute difference in effect size (Global – Local) effect size.

a) Effect size



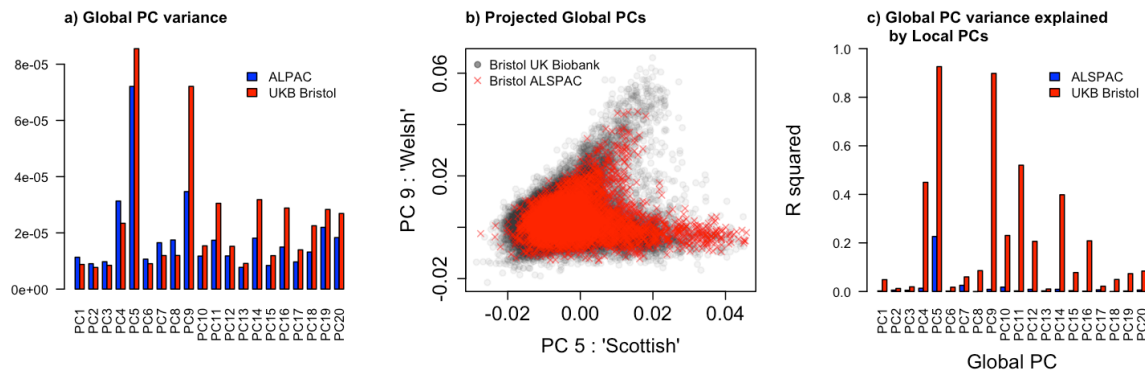
b) Difference in effect size



649



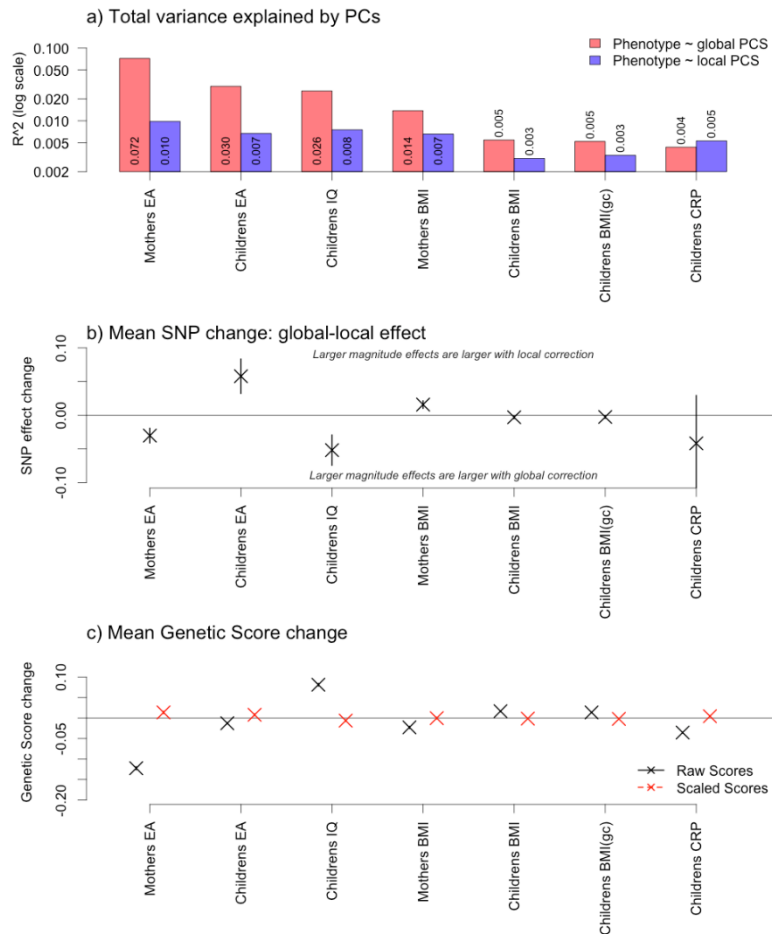
650 Figure 4. Population structure is lost in the ALSPAC cohort using local PCs.  
651 Local variation is lost when sample size reduces beyond a threshold as demonstrated by two studies  
652 in Bristol, the UK Biobank (N=27,503) and ALSPAC (N=7,927). a) Using global PCs constructed from  
653 UK Biobank variation, the two datasets have very similar genetic variation profiles across the first 20  
654 global PCs. b) Comparing PC5 (high values associated with Scottish ancestry) and PC9 (high values  
655 associated with Welsh ancestry) the structure is similar. c) When projecting local PCs into global PCs,  
656 the proportion of variance explained is high for Bristol UK Biobank but very low within ALSPAC, due  
657 to sample size.



658

659

660 Figure 5. Stratification correction affects SNP inference in the ALSPAC cohort  
 661 Stratification correction choice makes a measurable impact on inferences from the ALSPAC cohort.  
 662 a) Total variance in phenotype explained by global or local PCs (log scale). b-c) Weighted linear  
 663 regression coefficients for measuring local PC bias. The regression coefficient  $\hat{\beta}$  (and 95% confidence  
 664 interval) from  $\delta_i = z_{i,global} - z_{i,local} = \alpha + \beta z_i + \varepsilon_i$ , with  $z_i = (z_{i,global} + z_{i,local})/2$  and in b)  $z_i$  is  
 665 the SNP effect size for each GWAS. In c)  $z_i$  is the individual's Genetic Score, either raw (summing the  
 666 effect of each SNP present in the individual) or scaled to have mean 0 and s.d. 1 independently for  
 667 both GWAS.



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