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Title

Adjusting for principal components can induce spurious associations in genome-wide association studies in admixed populations

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Abstract

Principal component analysis (PCA) is widely used to control for population structure in genome-wide association studies (GWAS). It has been shown that the top principal components (PCs) typically reflect population structure, but deciding exactly how many PCs to include in GWAS regression models can be challenging. Often researchers will err on the side of including more PCs than may be actually necessary in order to ensure that population structure is fully captured. However, through both analytic results and application to TOPMed whole genome sequence data for 1,888 and 2,676 unrelated African American individuals from the Jackson Heart Study (JHS) and Chronic Obstructive Pulmonary Disease Genetic Epidemiology Study (COPDGene), respectively, we show that adjusting for extraneous PCs can actually induce spurious associations. In particular, spurious associations arise when PCs capture local genomic features, such as regions of the genome with atypical linkage disequilibrium (LD) patterns, rather than genome-wide ancestry. In JHS and COPDGene, we show that careful LD pruning prior to running PCA, using stricter thresholds and wider windows than is often suggested in the literature, can resolve these issues, whereas excluding lists of high LD regions identified in previous studies does not. We also show that the rate of spurious associations can be appropriately controlled in these data when we simply adjust for either the first PC or a model-based estimate of admixture proportions. Our work demonstrates that great care must be taken when using principal components to control for population structure in genome-wide association studies in admixed populations.

1 Introduction

Admixed populations such as African Americans and Hispanics/Latinos have historically been vastly underrepresented in genome-wide association studies (GWAS)[1,2,3,4,5,6]. Although this underrepresentation has many causes, some authors have cited the statistical challenges posed by ancestrally heterogeneous populations, such as admixed populations, as a possible contributing factor[1,2,3]. Considerable variability of inferred *global ancestry*—the genome-wide proportion of genetic material inherited from each ancestral population—has been observed in many studies of African American and Hispanic/Latino populations[7,8,9,10,11]. It has been widely documented that heterogeneous global ancestry, along with other types of population structure, can lead to spurious associations in genome-wide association studies[12,13,14,15]. These spurious associations arise due to the fact that global ancestry can confound the association between genotypes and a phenotype of interest, particularly when genetic variants are more frequent in some ancestral populations than in others and global ancestry has an effect on the trait through, for example, environmental differences across ancestral groups.

A number of methods for detecting and controlling for ancestral heterogeneity in genetic association studies have been proposed. Early approaches included restricting analyses to subsets of ancestrally homogeneous individuals[16], performing a genome-wide correction for test statistic inflation due to ancestral heterogeneity via *genomic control*[12], and using family-based designs[17]. More recently, approaches based on mixed models have been proposed[18,19,20]. These mixed model approaches use random effects to control for both close (e.g., due to family-based sampling) and distant (e.g., due to shared ancestry) relatedness across individuals. However, when studies do not include closely related individuals, a simpler approach is to include inferred global ancestry as a fixed effect in marginal regression models[13,21]. This fixed effects adjustment for global ancestry is currently used extensively throughout the literature, with global ancestry inferred using either model-based ancestry inference methods (e.g., `ADMIXTURE`[22]) or unsupervised dimension reduction techniques (e.g.,

principal component analysis[13]).

Model-based approaches for global ancestry inference model the probability of observed genotypes given unobserved ancestry and allele frequencies in each ancestral population[23,24,22,25]. Most often, these approaches are used to estimate global ancestry proportions, also known as *admixture proportions*, the estimated proportion of genetic material inherited by each individual from each ancestral population. Once estimated, these global ancestry proportions can then easily be included as covariates in GWAS models to adjust for ancestral heterogeneity. One of the challenges of using these model-based approaches to infer global ancestry is that the total number of ancestral populations usually needs to be pre-specified. In addition, many of these model-based approaches are *supervised*, requiring reference panel data from each ancestral population of interest to estimate allele frequencies. Furthermore, ancestry inference is typically conducted at a continental level (e.g., African versus European, rather than South European versus North European), so finer levels of population structure could be missed; recent efforts have considered global ancestry inference on a sub-continental scale[25,26].

Principal component analysis (PCA), on the other hand, is a widely-implemented unsupervised approach for inferring global ancestry that does not require reference panel data or pre-specification of the number of ancestral populations of interest and is capable of capturing sub-continental structure[27]. To infer global ancestry using PCA, we preform an eigenvalue decomposition of the genetic relationship matrix (GRM) $\hat{\mathbf{\Psi}} = \frac{1}{m}\mathbf{X}\mathbf{X}^\top$, where $\mathbf{X}$ is the $n \times m$ matrix of standardized gentoypes for n individuals at m single nucleotide variants (SNVs). The top eigenvectors, or *principal components* (PCs) of $\hat{\mathbf{\Psi}}$ tend to reflect global ancestry[28,29], so adjusting for PCs can be an effective approach for controlling for ancestral heterogeneity in genetic association studies[13]. In practice, however, determining the number of PCs needed to capture global ancestry can be difficult. Furthermore, it has been shown that PCs can sometimes capture features other than global ancestry, such as relatedness across samples[28,30], data quality issues[13,31], and/or small regions of the genome with un-

usual patterns of linkage disequilibrium (LD)[32,33]. To address this last issue, some authors have suggested running PCA on a reduced subset of SNVs, after first removing regions of the genome that are known to have high or long-range LD[33] and/or performing LD pruning[34,35]. However, these suggestions are not universally implemented, and the downstream implications of adjusting for PCs that capture features other than global ancestry are not fully understood.

In this paper, we investigate the impact of ancestral heterogeneity on genome-wide association studies in admixed populations. Through both simulation studies and analytic results, we provide new insight into when genetic association studies must adjust for global ancestry. In addition, we compare two approaches for adjusting for global ancestry, using model-based estimates of admixture proportions or principal components, and show that using PCs can actually induce spurious associations in GWAS. To conclude, we provide suggestions regarding best practice for appropriately controlling for ancestral heterogeneity in genetic association studies in admixed populations.

Reframe so that we're really focused on LD filtering –¿ move some of this from methods to intro

2 Material and Methods

2.1 Adjusting for ancestral heterogeneity in genome-wide association studies

To perform genome-wide association studies in samples of unrelated admixed individuals, we use marginal regression models, regressing the trait of interest on the genotype at each position across the genome. At a given position j, we quantify genotype g_{ij} as the number of copies (0, 1, or 2) of some pre-specified allele (e.g., the minor allele) carried by individual i at that position. Considering a quantitative trait y_i, we fit one linear regression model at

each position ($j = 1, \ldots, m$):

$$E[y_i \mid g_{ij}, \mathbf{z}_i] = \beta_0 + \beta_j g_{ij} + \boldsymbol{\beta}_z \mathbf{z}_i,$$

where $\mathbf{z}_i$ is a vector of additional covariates (e.g., potential confounding variables) that we want to include in the model. This linear regression model can be replaced with a logistic regression model in the case of a binary trait (e.g., disease status). In either case, we test for an association between the trait and genotype by testing the null hypothesis $H_0 : \beta_j = 0$ at each position $j = 1, \ldots, m$.

To adjust for ancestral heterogeneity, we include inferred global ancestry in the vector $\mathbf{z}_i$ of potential confounders in our regression models. We infer global ancestry using one of two techniques: model-based global ancestry inference of principal component analysis.

Note: should some of the following should perhaps go to the Introduction section instead?

2.1.1 Model-based global ancestry inference

Various model-based approaches have been developed for estimating global ancestry proportions in admixed populations. We represent global ancestry via the vector $\boldsymbol{\pi}_i = \begin{pmatrix} \pi_{i1} & \ldots & \pi_{iK} \end{pmatrix}^\top$, where π_{ik} denotes the genome-wide proportion of genetic material inherited by individual i from ancestral population k and $\sum_{k=1}^{K} \pi_{ik} = 1$. Note that the total number of ancestral populations, K, typically must be pre-specified, and the definition of global ancestry is typically restricted to the autosomes. Admixture proportions can be estimated directly using a program such as `ADMIXTURE`[22], or by calculating the genome-wide average local ancestry (i.e., $\hat{\pi}_{ik} = \frac{1}{2m} \sum_{j=1}^{m} a_{ijk}$), where local ancestry a_{ijk}—the number of alleles (0, 1, or 2) inherited by individual i from ancestral population k at position j—was first inferred using a program such as `RFMix`[36]. To adjust for ancestral heterogeneity, we include $K - 1$ of these estimated admixture proportions as covariates in our GWAS regression models:

$$E[y_i \mid g_{ij}, \hat{\boldsymbol{\pi}}_i] = \beta_0 + \beta_j g_{ij} + \beta_{\pi,1} \hat{\pi}_{i,1} + \cdots + \beta_{\pi,K-1} \hat{\pi}_{i,K-1}.$$

Many model-based global ancestry inference programs are supervised, requiring data from individuals from each ancestral population of interest to form a reference panel. However, some approaches such as `ADMIXTURE` can also be run without a reference panel.

2.1.2 Principal component analysis

Principal component analysis (PCA) is an unsupervised dimension-reduction technique that is widely used for inferring population structure in genetic studies, with a number of software programs available for running PCA on genotype or sequence data (e.g., `EIGENSTRAT`[13], `SNPRelate`[37], `PC-Air`[30]). To run PCA, we perform a singular value decomposition of the matrix of standardized genotypes (i.e., $\mathbf{X} = \mathbf{UDV}^\top$) or, equivalently, an eigenvalue decomposition of the genetic relationship matrix (i.e., $\mathbf{XX}^\top = \mathbf{UD}^2\mathbf{U}^\top$). The top principal components ($\mathbf{u}_1, \mathbf{u}_2, \dots$) typically capture global ancestry[28,29]. To adjust for ancestral heterogeneity, we choose some number of principal components, P, needed to capture global ancestry (typically $1 \leq P << n$) and include those PCs as covariates in our GWAS regression models:

$$E[y_i \mid g_{ij}, u_{i1}, \dots, u_{iP}] = \beta_0 + \beta_j g_{ij} + \beta_{u1} u_{i1} + \cdots + \beta_{uP} u_{iP}.$$

A number of techniques have been proposed for selecting the number of PCs, P, including formal significance tests based on Tracy-Widom theory[28,13], examining the proportion of variance explained by each PC[38], comparing PCs to self-reported ancestry[11], and/or keeping PCs that are significantly associated with the trait[39,40].

[... also some people just include how ever many PCs another paper included ...]

[... Mention that many people will include more than necessary? ...]

[... state what approach we take in this paper to choosing P ...]

2.1.3 Variant- and sample-level filtering

It is often recommended that filtering be performed at the variant and/or sample level prior to inferring global ancestry. Prior work has shown that both model-based estimates of

global ancestry [... cite Tim's GAW paper ...] and principal components[30,13,28] [... check patterson, maybe add more refs: Price 2010 ...] can reflect family structure and/or cryptic relatedness rather than global ancestry when a sample includes related individuals, but restricting analyses to a subset of unrelated individuals (e.g., using the iterative procedure proposed by[41]) can circumvent that issue. At the variant level, it is common to perform filtering based on minor allele frequency [... find references ...], as prior work has shown that methods such as `EIGENSTRAT` can perform poorly when applied to rare variants[42].

Other variant-level filters have been recommended to address the sensitivity of model-based and PCA approaches to the presence of linkage disequilibrium (LD). This can include *LD pruning*, using a program such as `PLINK` [... CITE ...] to remove variants that are "highly" correlated (e.g., pairwise-correlation $r^2 > 0.2$) with nearby variants (e.g., within a window of size ??) [...[22], ADD MORE ...], and/or excluding regions of the genome that are known to have extensive, long-ranging, or otherwise unusual patterns of LD [... CITE ...]. A list of previously-identified high LD regions is provided in Appendix ??. [... say something about how not everyone does this, it's not always clear which parameters should be used, and/or much of this work has been performed in European pop and not clear what should be done in admixed pop ...]

[... Add missing rates (SNPs and people) here? Or just frame as QC step?) ...]

2.2 Simulation study using TOPMed whole genome sequence data

Are we sure we want to use TOPMed? Or should we switch back to WHI?

We implement a simulation study using whole genome sequence data from the Trans-Omics for Precision Medicine (TOPMed) program to compare different approaches for adjusting for ancestral heterogeneity and explore the impact of different variant-level filtering choices, particular with respect to linkage disequlibrium.

2.2.1 TOPMed whole genome sequence data

TOPMed is [... describe TOPMed ...]. Whole genome sequence data for contributing TOPMed studies is available on dbGaP. We focus on two such studies, the Jackson Heart Study (JHS) (accession number: phs000964) and the Chronic Obstructive Pulmonary Disease Genetic Epidemiology Study (COPDGene) (accession number: phs000951).

- describe TOPMed
- describe sequencing methods
- how many samples in each study

2.2.2 Quality control

We process the sequence data for quality control, keeping only those variants that are bi-allelic, have a minor allele count of at least one, and pass standard variant filters (Mendelian or duplicate genotype discordance $< 3/5\%$, Hardy-Weinberg Equilibrium p-value $> 1 \times 10^{-6}$, etc.). [... Add filter for missing rates! ...] After all exclusions, we are left with ??? and ??? variants in JHS and COPDGene, respectively.

After variant-level filtering, we then use the iterative procedure suggested by Conomos et al.[11] and implemented in the TOPMed analysis pipeline to identify a subset of ?? mutually unrelated individuals. [... more details about iterative procedure; e.g., sing a kinship threshold of 0.044 ← double-check (i.e., excluding first, second, and third degree relatives) ...] Next, we run an unsupervised `ADMIXTURE` analysis with $K = 2$ and $K = 3$ and plot inferred global ancestry proportions to identify admixed (African American) and non-admixed (European) individuals. [... more details about thresholds used ...] After sample-level filtering, ??? and ??? individuals remain in JHS and COPDGene, respectively.

QC for JHS (dbgap accession phs000964):

- filtering

  - bi-allelic SNPs
  - minor allele count at least 1
  - pass variant filters (in VCFs that were downloaded from dbgap): overlaps with SNP, overlaps with indel, overlaps with VNTR, failed SVM filter, high (3/5% or more) mendelian or duplicate genotype discordance, excess heterozygosity with HWE p-value ¡ 1e-6

- merge the two subsets (cg1 and cg3)
- convert from VCF to GDS
- remove close relatives
  - run king (LD r threshold: 0.32, LD window size: 10, MAF threshold: 0.01, exclude PCA corr: TRUE, build: hg19)
  - run PC-AiR
  - run PCRelate
  - run PC-AiR again
  - run PCRelate again
- find African Americans
  - run stricter LD pruning (MAF = 0.01, window size = 0.5, rsq = 0.01, regions = TRUE, build 37)
    - List of regions stored here: `/projects/browning/brwnlab/kelsey/spurious_assoc/highl`
  - convert GDS to BED
  - run ADMIXTURE (K = 2 and K = 3)
  - plot proportions
  - exclude 40 people inferred to be 100% European; left with 1888

QC for COPDGene (phs000951):

- filtering
  - bi-allelic SNPs
  - minor allele count at least 1
  - pass filtering (from GDS annotation info, I inferred this to include: variant located in centromeric region, variant failed SVM filter, mendelian or duplicate genotype discordance is high (3/5% or more), excess heterozygosity in chrX in males, excess heterozygosity with HWE p-value ¡ 1e-6)
- convert from VCF to GDS
- remove close relatives
  - run king (LD r threshod = 0.32, LD window size = 10, MAF threshodl = 0.01, exclude PCA corr = TRUE, build = hg38); regions = see table below
  - run PCAiR
  - run PCRelate
  - run PCAiR again
- find African Americans
  - run stricter LD pruning (exclude PCA corr regions = TRUE, build = hg38, LD R threshold = 0.1, LD window size = 10, MAF threshold = 0.01); regions = `/projects/browning/brwnlab/kelsey/spurious_assoc/highLD_regions/`
  - convert from GDS to PLINK
  - run ADMIXTURE with K = 2 and K = 3

name	chrom	start.base	end.base	comment
2q21	2	129883530	140283530	LCT
HLA	6	24092021	38892022	includes MHC
8p23	8	6612592	13455629	inversion
17q21.31	17	40546474	44644684	inversion

Table 1: TOPMed hg19 high corr regions

name	chrom	start.base	end.base	comment
2q21	2	129125957	139525961	LCT
HLA	6	24091793	38924246	includes MHC
8p23	8	6755071	13598120	inversion
17q21.31	17	42394456	46567318	inversion

Table 2: TOPMed hg38 high corr regions

  - plot proportions and use cut-off of 30% to identify (and then remove) Europeans : Parker et al. 2014 "Admixture mapping identifies a quantitative trait locus associated with..." `https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4190160/` (reduced from 8406 to 2676)

2.2.3 Genetic ancestry inference

- ADMIXTURE
  - JHS: ran with both K = 2 and K = 3
  - COPDGene: ran with both K = 2 and K = 3
  - unsupervised for both
- PCA using SNPRelate
- what filtering was performed, and how many variants left after filtering
  - JHS, ADMIXTURE: see above
  - JHS, PCA: exclude regions (TRUE/FALSE), r-squared (1, 0.1, 0.2, 0.05), window size (0, 0.5, 10), and MAF (0, 0.01)

    - no filtering: FALSE-1-0-0
    - MAF filtering: FALSE-1-0-0.01
    - exclude but no prune: TRUE-1-0-0.01
    - prune but no exclude: FALSE-0.1-0.5-0.01 and FALSE-0.1-10-0.01 and FALSE-0.2-0.5-0.01 and FALSE-0.05-0.5-0.01
    - prune and exclude: TRUE-0.1-0.5-0.01 and TRUE-0.1-10-0.01 and TRUE-0.2-0.5-0.01 and TRUE-0.05-0.5-0.01
  - COPD, ADMIXTURE: see above
  - COPD, PCA: exclude regions (TRUE/FALSE), r-squared (1, 0.1, 0.2, 0.05), window size (0, 0.5, 10), MAF (0, 0.01)
    - no filtering: FALSE-1-0-0
    - MAF filtering: FALSE-1-0-0.01
    - exclude but no prune: TRUE-1-0-0.01
    - prune but no exclude: FALSE-0.1-0.5-0.01, FALSE-0.1-10-0.01, FALSE-0.2-0.5-0.01, FALSE-0.05-0.5-0.01
    - prune and exclude: TRUE-0.1-0.5-0.01, TRUE-0.1-10-0.01, TRUE-0.05-0.5-0.01, TRUE-0.2-0.5-0.01
  - COPD, also ran SNPRelate on Europeans with different levels of filtering (FALSE-0.1-0.5-0.01, FALSE-0.2-0.5-0.01, FALSE-1-0-0.01, FALSE-1-0-0, TRUE-0.1-0.5-0.01, TRUE-0.2-0.5-0.01, TRUE-1-0-0.01)

2.2.4 Evaluating population structure adjustment approaches

- plot PCs vs global ancestry proportions
- plot SNP loadings
- simulating traits (effect sizes, choice of causal SNPs)

  - find loading peaks from "naive" approach
  - simulate trait that is beta * x + rnorm(0, 1), where beta = 1 or 2 and x = genotype at one of the peaks
- running GWAS
  - for each of 188*2 simulated phenotypes
  - for each set of PCs
  - including 1, 4, or 10 PCs
- defining spurious associations

3 Results

3.1 Ancestral heterogeneity in TOPMed African American samples

- quickly summarize ancestral heterogeneity (barplots of ADMIXTURE proportions)

3.2 Confirming the importance of adjusting for population structure

- show an example manhattan plot with no adjustment
- compare average number of spurious associations
- tie in theoretical results

3.3 Comparing different approaches for adjusting for population structure

Part 1: how does FWER compare?

- manhattan plots for one or two simulated traits
- overall summary of rejection rates
- is it appropriate to use same significance threshold for all?

Part 2: how does rate of spurious associations compare? (and alpha-adjusted spurious assoc?)

- manhattan plots for one or two traits
- overall summary of rejection rates

Part 3: why is this happening?

- are admixture proportions and PCs capturing similar information?
  - correlation between PCs and admixture proportions (PC1 highly correlated with admix prop)
  - correlation between PCs and genotypes (without pruning, later PCs highly correlated with genotypes in small regions)
- mathematical results

4 Discussion

Global ancestry = confounder

- Summarize conditions under which global ancestry is a confounder
- Relate to current understanding in literature

Be careful with PCs!

- Summarize conditions under which PCs can be problematic

- Relate to current understanding in literature
- Relate to concept of collider bias
- Suggested diagnostics

5 Appendices

5.1 Regions Removed Prior to PCA

- a list of all "high-LD" regions removed prior to running PCA

5.2 Mathematical Derivations

- theoretical results
- proofs
- simulations validating theory

Supplemental Data

Supplemental Data include [... ?? ...] figures and [... ?? ...] tables.

Declaration of Interests

The authors declare no competing interests.

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Web Resources

GitHub Repository: lists of regions to exclude, code for LD pruning, excluding, and plotting loadings

Data and Code Availability

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Figure Titles and Legends

Tables