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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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If we use WHI data, do we need to add Alex as well?

Abstract

NEEDS UPDATING

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

Considerable variability in global ancestry—the genome-wide proportion of genetic material inherited from each ancestral population—has been observed in many studies of admixed populations such as African Americans and Hispanics/Latinos^{1,2,3,4,5}. It has been widely documented that heterogeneous global ancestry, as with other types of population structure, can lead to spurious associations in genome-wide association studies^{6,7,8,9}. In fact, some authors have even cited the ancestral heterogeneity of admixed populations, and the statistical challenges it poses, as one of many reasons why these populations have been historically underrepresented in genome-wide association studies (GWAS)^{10,11,12,13,14}. Spurious associations can arise in GWAS in ancestrally heterogeneous populations when global ancestry confounds the association between genotypes and the phenotype of interest (Figure 1). This confounding occurs when the genetic variant being tested differs in frequency across ancestral populations (i.e., global ancestry is associated with genotype) and global ancestry also has an effect on the phenotype via, for example, environmental factors or causal loci elsewhere in the genome that differ in frequency across ancestral groups.



Figure 1: Global ancestry (π) confounds the association between the genotype at position j (g_j) and the phenotype of interest (y) if ancestry is associated with both the genotype (e.g., the allele frequencies differ across the ancestral populations) and the phenotype (e.g., there are environmental or other factors that affect the phenotype and differ across the ancestral populations).

A number of methods for detecting and controlling for ancestral heterogeneity in ge-

netic association studies have been proposed. Early approaches included restricting analyses to subsets of ancestrally homogeneous individuals¹⁵, performing a genome-wide correction for test statistic inflation due to ancestral heterogeneity via *genomic control*⁶, and using family-based designs¹⁶. More recently, approaches based on mixed models have been proposed^{17,18,19}, using 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. 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^{7,20}. 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., `frappe`²¹, `STRUCTURE`²², `ADMIXTURE`²³, `RFMix`²⁴) or principal component analysis (e.g., `EIGENSTRAT`⁷, `SNPRelate`²⁵, `PC-AiR`²⁶).

Principal component analysis (PCA) is a widely-implemented unsupervised approach for inferring global ancestry. Advantages of this approach are that it does not require reference panel data or pre-specification of the number of ancestral populations of interest, and it is capable of capturing sub-continental structure²⁷. To infer global ancestry using 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., $\mathbf{XX}^\top = \mathbf{UD}^2\mathbf{U}^\top$). It has been shown that top eigenvectors, or *principal components* (PCs), $\mathbf{u}_1, \mathbf{u}_2, \dots$ tend to reflect global ancestry^{28,29}. To adjust for ancestral heterogeneity in genome-wide association studies, we choose some number of PCs to include as covariates in our GWAS regression models.

Determining the number of PCs needed to capture global ancestry is non-trivial. Numerous techniques have been proposed, including formal significance tests based on Tracy-Widom theory^{28,7}, examining inflation factors^{30,5} and/or the proportion of variance explained by each PC^{31,30,5}, comparing PCs to self-reported race/ethnicity⁵, and keeping PCs that are significantly associated with the trait^{32,33}. Typically, the number of PCs selected is on the order

of one to ten³⁴, but in practice it is not uncommon to see applications in which more many more PCs are used—more even than may actually be necessary to capture global ancestry. This could be due in part to work that has suggested that including higher-order PCs can provide the safeguard of removing “virtually all stratification”³⁵ at the cost of perhaps only “subtle” decreases in power³⁶.

Another challenge that can arise in using PCA to adjust for ancestral heterogeneity involves ensuring that PCs actually reflect global ancestry and not some other features or artifacts of the data. Prior work has shown that PCs can capture relatedness across samples^{28,9,37,26}, array artifacts or other data quality issues^{28,7,9,38}, and/or small regions of the genome with unusual patterns of linkage disequilibrium (LD)^{28,7,39,40,41,9,38,42,43,37,44}. To address this last issue, some authors have suggested running PCA on a reduced subset of variants after first performing *LD pruning*, using a program such as PLINK⁴⁵ to remove variants that are in “high” LD (e.g., pairwise-correlation $r^2 > 0.2$) with nearby variants^{39,46,27,47,48,49,38,43,37,50,26,30,51,5,33}, and/or excluding regions of the genome that are known to have extensive, long-ranging, or otherwise unusual patterns of LD^{39,46,27,41,49,38,31,5}. A list of these previously-identified high LD regions and references that recommend their exclusion are provided in Table 1.

The above-cited suggestions regarding LD pruning and filtering are not universally implemented and the downstream implications of adjusting for PCs that capture features other than global ancestry are not fully understood. Furthermore, much of this work was conducted in populations of European ancestry, so recommendations on how best to implement principal component-based adjustment for ancestral heterogeneity in admixed populations are lacking. In this paper, we investigate the impact of LD filtering and pruning choices, as well as choices of the number of principal components to include in analyses, on genome-wide association studies in admixed populations. We conduct simulation studies using whole genome sequence data for African American individuals in the Trans-Omics for Precision Medicine (TOPMed) project and provide analytic results to show that including too many

Chr	Start (bp)	End (bp)	References
1	48000000	52060567	49,41,38
2	85941853	100500000	49,41,38
2	129600000	140000000	41,27,38,31,5,52
2	182882739	190000000	49,41,38
3	47500000	50000000	49,41,38
3	83500000	87000000	49,41,38
3	89000000	97500000	41,38
3	163100000	164900000	52
5	44000000	51500000	46,49,41,38
5	98000000	100500000	41,38
5	129000000	132000000	49,41,38
5	135500000	138500000	41,38
6	23800000	39000000	46,49,41,27,38,31,5,52
6	57000000	64000000	49,41,38
6	140000000	142500000	49,41,38
7	55000000	66193285	49,41,38
8	6300000	13500000	46,49,41,27,40,38,31,5,52
8	43000000	50000000	49,41,38
8	112000000	115000000	49,41,38
10	37000000	43000000	49,41,38
11	45000000	57000000	46,41,38
11	87500000	90500000	49,41,38
12	33000000	40000000	49,41,38
12	109500000	112021663	41,38
14	46600000	47500000	52
17	37800000	42000000	27,5
20	32000000	34500000	49,41,38

Table 1: Regions of the genome with high, long-range, or otherwise unusual patterns of linkage disequilibrium (LD) that are often recommended for exclusion prior to running PCA. This list of regions was generated on the basis of an extensive literature review. Start and end physical (base pair) positions are provided with respect to genome build 36. Also available for download (in builds 36, 37, or 38) at <https://github.com/kegrinde/PCA/>. **UPDATE TO REFLECT WHI ANALYSES**

PCs can actually induce spurious associations in GWAS, particularly when those extraneous PCs capture local genomic features rather than genome-wide ancestry. — ADD WHI To conclude, we provide suggestions regarding best practice for appropriately controlling for ancestral heterogeneity in genome-wide association studies in admixed populations.

2 Material and Methods

ADD WHI — see dissertation

2.1 TOPMed Whole Genome Sequence Data

The Trans-Omics for Precision Medicine (TOPMed) Whole Genome Sequencing Project is an ongoing project sponsored by the National Heart, Lung, and Blood Institute (NHLBI) that is working to collect and analyze whole-genome sequences, other 'omics data, and rich phenotypic information for over 100,000 individuals from diverse backgrounds. Data are periodically released on dbGaP for analysis by the broader scientific community. Our analysis uses data from *freeze 4*, released in 2017, and *freeze 5b*, released in 2018. These two freezes include samples from a large number of contributing studies. We focus on two such studies: the Jackson Heart Study (JHS) (accession number: phs000964) and the Genetic Epidemiology of Chronic Obstructive Pulmonary Disease Study (COPDGene) (accession number: phs000951). In total, the freeze 4 JHS dataset includes 3,406 African American individuals and the freeze 5b COPDGene dataset includes 8,742 African American and European American individuals.

For TOPMed freezes 4 and 5b, high coverage ($\approx 30X$) whole genome sequencing was performed by several sequencing centers. Variant discovery and genotype calling was performed by the TOPMed Informatics Resources Center (IRC) using the GotCloud pipeline⁵³. Quality control (QC) was performed by the sequencing centers, IRC, and TOPMed Data Coordinating Center, and only those samples and variants that passed these stages of QC are included

in the VCF downloaded from dbGaP. Details on TOPMed sequencing and QC methods are available in Taliun et al.⁵⁴ and on the TOPMed website: <https://topmed.nhlbi.nih.gov/> datasets.

2.2 Additional Quality Control and Filtering

Prior to principal component analysis, we perform additional variant- and sample-level filtering. We use `bcftools`⁵⁵ to remove indels and otherwise restrict our analyses to biallelic single nucleotide variants (SNVs). We also remove variants with low minor allele frequency (< 1%) and/or high rates of missing calls (> 1%) — STILL NEEDS TO BE IMPLEMENTED. After this filtering, a total of ??? SNVs remain in JHS and ??? SNVs remain in COPDGene.

At the sample level, we use the iterative procedure proposed by Conomos et al.⁵⁶ and implemented in the TOPMed Analysis Pipeline to identify a subset of mutually unrelated individuals using a kinship threshold of 0.044 (third degree relatives). We also perform an unsupervised ADMIXTURE²³ analysis with both $K = 2$ and $K = 3$ to identify admixed (African American) and non-admixed (European American) individuals; we restrict remaining analyses to admixed individuals only. Prior to both of these analyses we implement LD pruning/filtering as recommended in their respective user manuals. After exclusions, a total of ??? and ??? unrelated African Americans remain in JHS and COPDGene, respectively.

2.2.1 LD-Based Filtering

In addition to the filtering described above, we also implement different types of LD-based filtering. [... Describe the different types of LD-based filtering we compared (see below). ...] These analyses are also compared to a *naive* analysis that did not perform any LD-based filtering. The number of variants that remain after each type of filtering is presented in Table 2.

- Exclude

- None
- Lit Review (Table 1)
- Auto-Detect ([... using Prive package — implement or skip for now?? ...])
- Prune
 - None
 - Default 0.2
 - Stricter 0.1
 - [... Stricter 0.05 ??? ...]
 - [... Different window size ??? ...]

	Naive	Exclude	Prune	Stricter Prune	Exclude + Stricter Prune
JHS	[... ? ...]	[... ? ...]	[... ? ...]	[... ? ...]	[... ? ...]
COPDGene	[... ? ...]	[... ? ...]	[... ? ...]	[... ? ...]	[... ? ...]

Table 2: Single nucleotide variants that remained in Jackson Heart Study (JHS) and Genetic Epidemiology of COPD (COPDGene) datasets after varying levels of LD-based filtering.

- 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)

2.3 Principal Component Analysis

We use the `SNPRelate` package in R to run principal component analysis using each of the subsets of SNVs described in Section 2.2. For each set of principal components, we also use the `SNPRelate` package to assess the contribution of each variant to each PC by calculating and plotting the correlation between genotypes and PCs. [... also look at loadings? shouldn't this give us the same picture as corr? ...]

2.4 Simulation Study to Investigate Rates of Spurious Associations

We implement a simulation study to explore the impact of different variant-level filtering choices, particularly with respect to linkage disequilibrium, on rates of spurious associations in genome-wide association studies using models that adjust for ancestral heterogeneity using principal components.

2.4.1 Simulated Traits

[...]

- find loading peaks from "naive" approach
- simulate trait that is $\text{beta} * \mathbf{x} + \text{rnorm}(0, 1)$, where $\text{beta} = 1$ or 2 and \mathbf{x} = genotype at one of the peaks

...]

2.4.2 GWAS Models

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 ($j = 1, \dots, m$):

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

where \mathbf{z}_i is a vector of additional covariates (e.g., potential confounding variables) that we want to include in the model. We test for an association between the trait and genotype by

testing the null hypothesis $H_0 : \beta_j = 0$ at each position $j = 1, \dots, m$.

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. [...
describe which models we compare ...]

[...]

- for each of 188*2 simulated phenotypes
- for each set of PCs
- including 1, 4, or 10 PCs

...]

2.4.3 Evaluation

[... defining spurious associations ...]

2.5 Code and Data Availability

All code and data used throughout this paper are publicly available online:

- TOPMed Sequence Data: <https://www.ncbi.nlm.nih.gov/gap/>
- Code: <https://github.com/kegrinde/PCA>

3 Results

3.1 Ancestral heterogeneity in admixed populations

Inferred admixture proportions for three samples of African American individuals are presented in Figure 2.

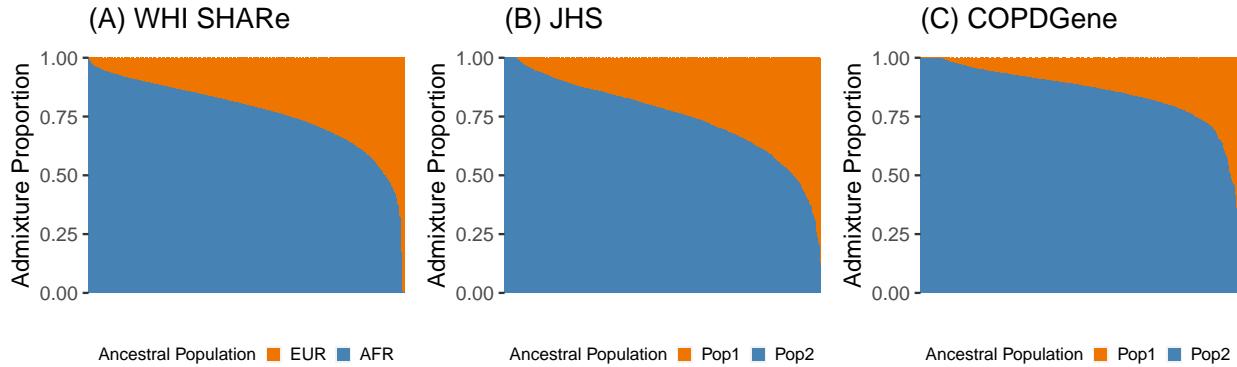


Figure 2: Barplots of estimated admixture proportions in WHI SHARe (A), TOPMed JHS (B), and TOPMed COPDGene (C) African Americans.

In WHI SHARe African Americans, we compared admixture proportion estimates from a variety of model-based techniques. Figure 2 presents admixture proportions estimated as genome-wide average local ancestry, using local ancestry calls from **RFMix**. [Move to methods?](#) We also ran supervised and unsupervised **ADMIXTURE** analyses with two ancestral populations ($K = 2$). All three sets of admixture proportions were highly correlated (pairwise Pearson correlation > 0.998), so we decided to use only the local ancestry based admixture proportion estimates for the remainder of our analyses.

In TOPMed samples, we performed unsupervised **ADMIXTURE** analyses with varying numbers of ancestral populations. Figure 2 presents results with $K = 2$. Although these analyses were unsupervised, based on prior studies of admixture in African Americans, and in comparison to the distribution of admixture proportions seen here in WHI SHARe, we believe that the ancestral population colored orange (Pop1) in Figure 2 corresponds to European ancestry and the population colored in blue (Pop2) corresponds to African ancestry.

[This paragraph could be moved to methods?](#) Note that we performed some filtering of individuals prior to presenting these barplots of estimated admixture proportions. Our work focuses on genetic association studies in admixed populations, but the COPDGene study includes both African Americans and European Americans. We do not have self-identified or reported race/ethnicity information for these samples from dbGaP, so we instead used

inferred admixture proportions to identify and restrict our attention to individuals with at least 29.5% African ancestry. The choice of threshold follows from the results reported by Parker et al.⁵⁷, showing that African Americans in the COPDGene study have inferred proportions of African ancestry ranging from 29.5% and above. After filtering, 2676 individuals remain. In addition, although JHS is known to focus on African American individuals, we did see some individuals inferred to have 100% European ancestry in that sample. These individuals were excluded from further analyses, leaving a total of 1888 admixed samples.

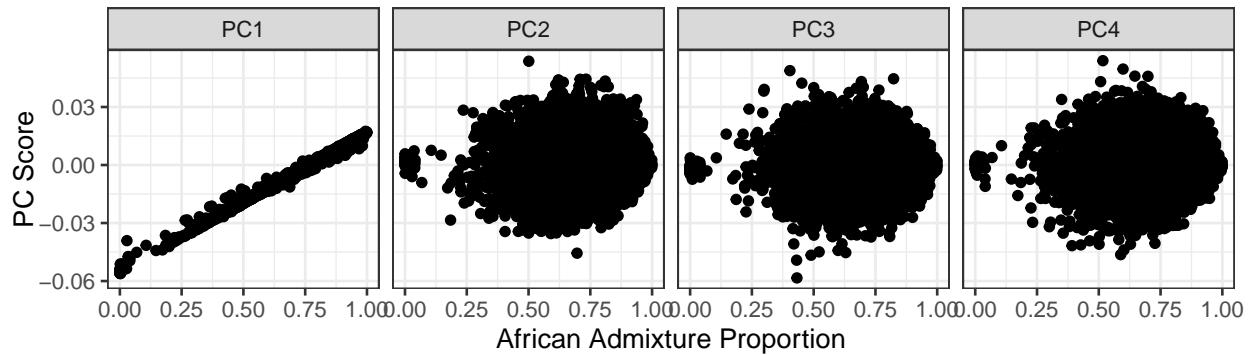
In all three samples, we observe considerable variability in the relative proportions of African and European ancestry across individuals. This ancestral heterogeneity motivates the need to carefully adjust for global ancestry in genome-wide association studies in these, just as in other, admixed samples.

- Add WHI SHARe Hispanic Americans? (check with Tim)
- Possible supplemental figure: JHS and COPDGene with $K = 3$ (and/or $K = 4$ for COPDGene)
- Possible supplemental figure: JHS and COPDGene barplots before filtering

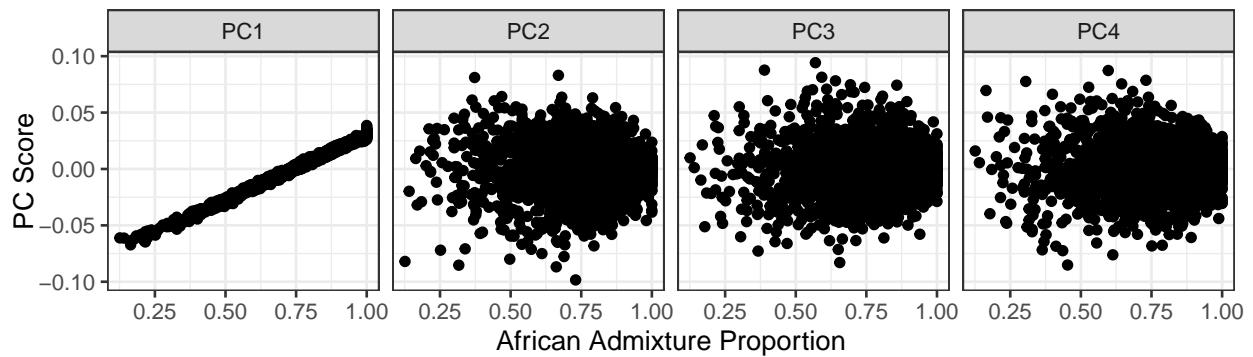
3.2 Initial PC captures global ancestry

In an African American population, we might expect that only one principal component is needed to capture ancestral heterogeneity, at least with respect to differences in the relative proportion of African and European continental ancestry. Comparing model-based admixture proportions to principal components in WHI SHARe, JHS, and COPDGene confirms this hypothesis. In all three samples of African Americans, the first principal component is highly correlated with the inferred proportion of African ancestry, while later PCs show very little correlation with genome-wide continental ancestry (Figure 3). We observe similar patterns of correlation between PCs and inferred admixture proportions regardless of the

(A) WHI SHARe



(B) JHS



(C) COPDGene

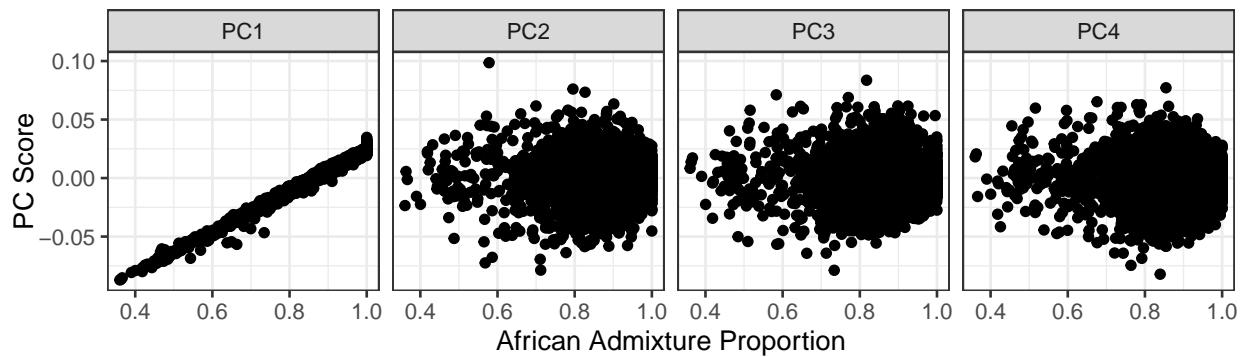


Figure 3: Scatterplots of estimated African admixture proportions versus the first four PCs in WHI SHARe (A), TOPMed JHS (B), and TOPMed COPDGene (C) African Americans. Here we consider PCs that were generated without any LD-based filtering or pruning.

type of LD filtering (or lack therof) performed prior to running PCA (Supplemental Figure 8).

3.3 Later PCs may capture local genomic features

As we see in Figure 3, in African American samples the first principal component seems to be capturing global ancestry, whereas later PCs are not. While it is possible that these higher-order principal components may be capturing sub-continental structure that is not captured by the model-based admixture proportions, we see in many cases that these later PCs are actually capturing local genomic features rather than genome-wide ancestry. This is evident upon inspection of *SNP loadings*, which represent the contribution of each variant to each principal component, or in investigating the correlation between principal component scores and the original genotypes. For example, Figure 4 presents the correlation between principal components and genotypes in JHS and COPDGene African Americans when PCs are generated without any prior LD-based pruning or filtering. We see that variants across the genome are contributing relatively equally to the first principal component, whereas the second, third, and fourth PCs are driven more-so by variants on just a select number of chromosomes. For example, the second PC is particularly highly correlated with variants on chromosomes 6 and 8—and less so with variants on 2, 3, and 11—in JHS. We see similar patterns, although with peaks on different combinations of chromosomes, in COPDGene (Figure 4B) and WHI SHARe African Americans (leftmost column of Figure 5). The peaks in these genotype-PC correlation plots indicate that those PCs are primarily capturing variation at a handful of positions along the genome rather than genome-wide global ancestry. Note that these patterns differ slightly from what has previously been observed in European populations: in particular, in European populations a PC might capture variation on a single chromosome (e.g., see Supplemental Figure 12), whereas here in these admixed populations we see PCs driven by contributions from variants across several chromosomes.

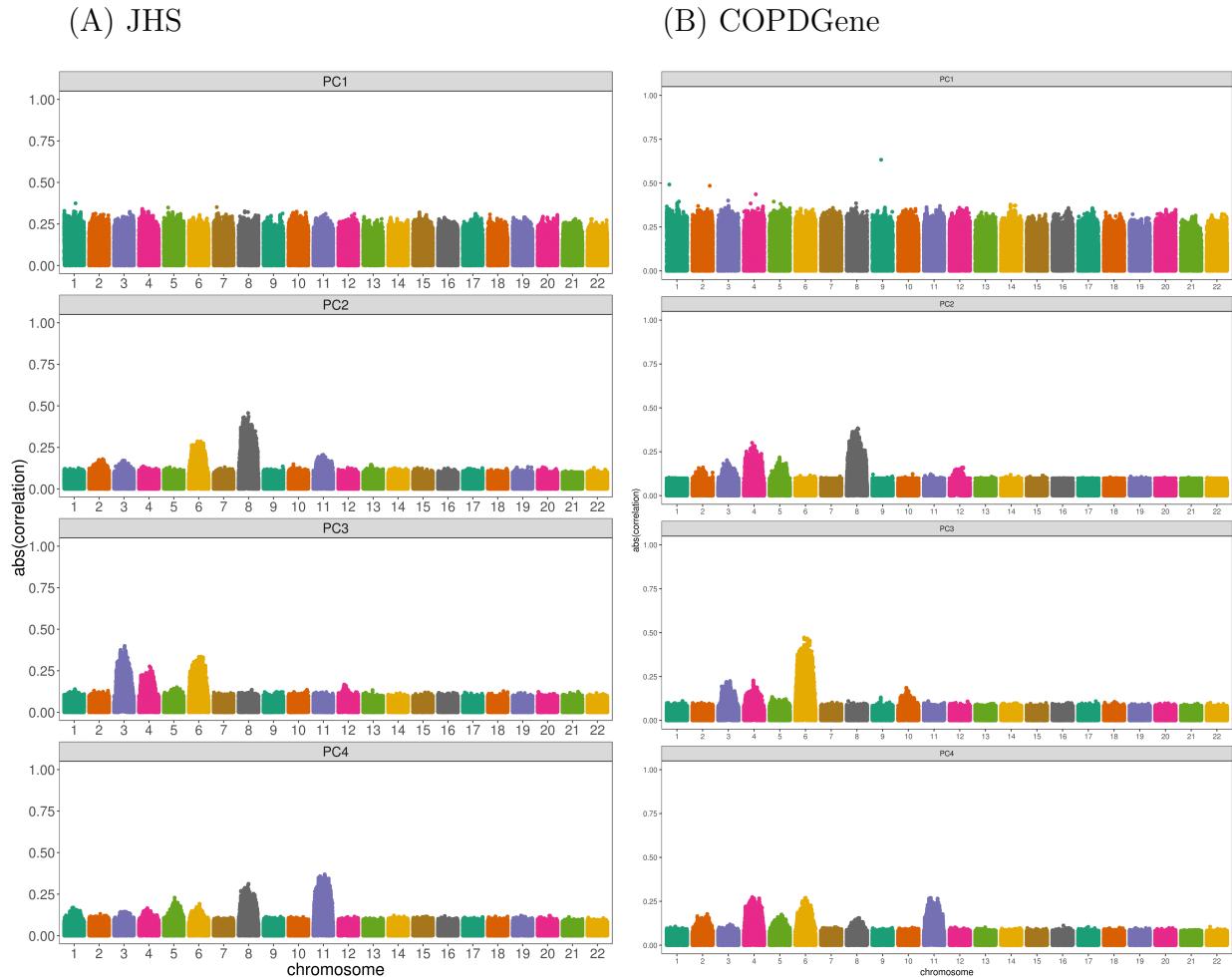


Figure 4: Correlation between naively generated PCs (i.e., PCs that were constructed without any prior LD-based filtering or exclusions) and genotypes in JHS and COPDGene African Americans. Each panel plots the absolute value of the correlation between principal components and genotypes (on the y-axis) versus the position along the genome (x-axis). Panels are organized vertically according to which PC is being investigated (1, 2, 3, 4) and horizontally according to the sample (A: JHS, B: COPDGene). Peaks in this plot indicate that a variant has a larger *loading*, i.e., a larger contribution to that principal component.

3.4 Impact of LD pruning

Previous authors have suggested that this phenomenon of principal components capturing local genomic features arises due to high or otherwise unusual patterns of linkage disequilibrium among variants; as a result, they recommend that variants in high LD with one another be removed prior to running PCA. Following these recommendations, we compare the set of principal components based on all variants to PCs generated after first removing regions of the genome known to have high LD (Table 1), performing LD pruning, or both. The remaining panels of Figure 5 show the correlation between genotypes and these other sets of PCs in WHI SHARe African Americans. When we exclude previously-identified high LD regions before running PCA (the second column of Figure 5), the pattern of *which* SNPs are driving PCs 2–4 changes, but the issue of PCs capturing local genomic features has not been resolved. However, after LD pruning with an r^2 threshold of 0.1 and a window size of 0.5 Mb (third column), we now see similar patterns with PCs 2–4 as we do with the first principal component — all variants are now contributing relatively equally to each PC. If we then also remove previously-identified high LD regions in addition to performing LD pruning (right-most column), the patterns of correlation between PCs and genotypes are indistinguishable from running LD pruning alone. Note that the thresholds for LD pruning that we use here ($r^2 < 0.1$) are stricter than the default for many software programs ($r^2 < 0.2$): if we use this default r^2 threshold, we see improvement for the second and third principal components, but the fourth continues to capture local genomic features on a small number of chromosomes (Supplemental Figure 9). Similar patterns are observed in JHS and COPDGene.

3.5 Adjusting for PCs that capture local genomic features can induce spurious associations

We have demonstrated that, especially without strict LD pruning, principal components can capture local genomic features rather than global ancestry in admixed populations. However,

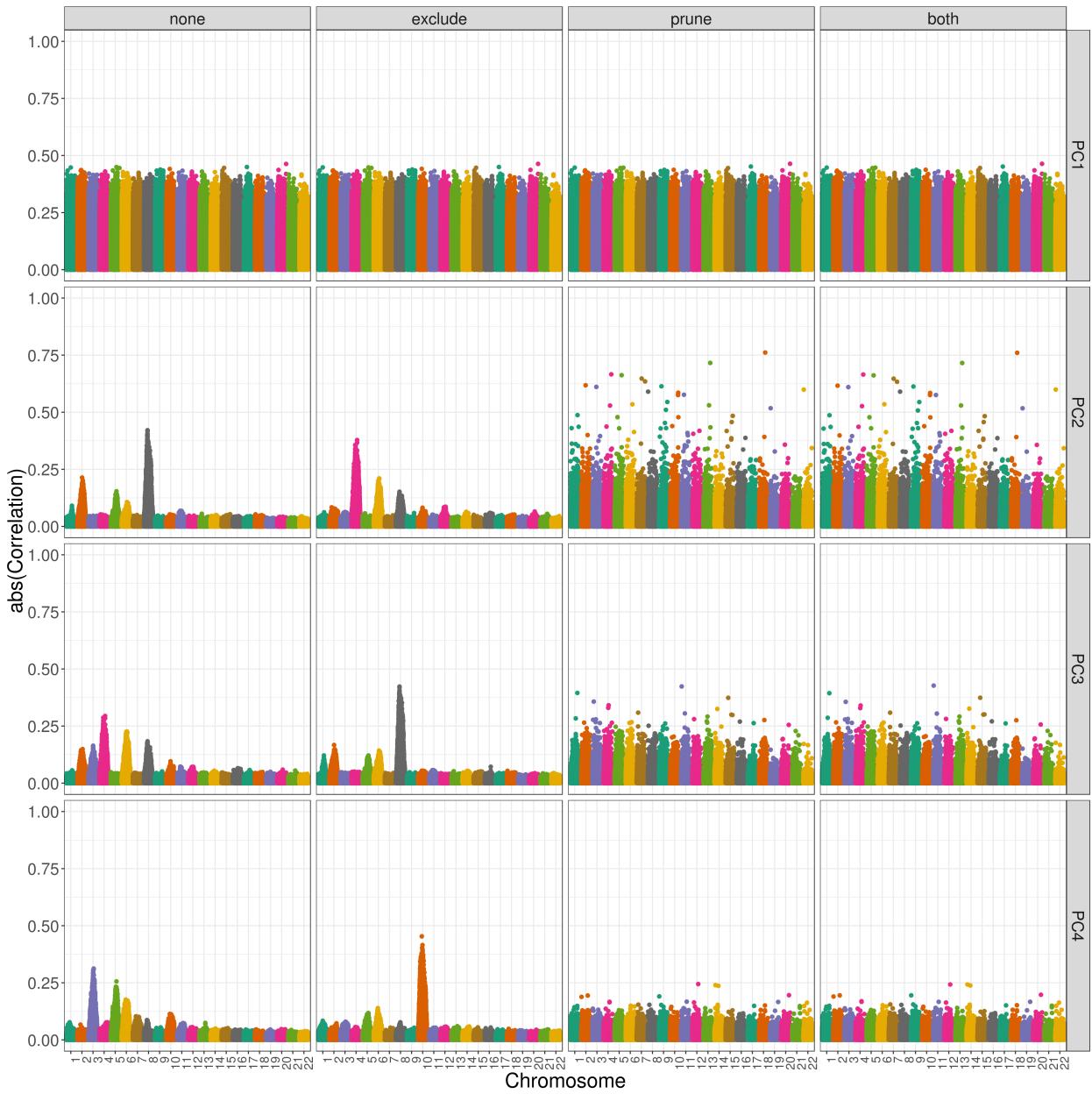


Figure 5: Correlation between PCs and genotypes in WHI SHARe African Americans with different choices of pre-processing. Each panel plots the absolute value of the correlation between principal components and genotypes (on the y-axis) versus the position along the genome (x-axis). Panels are organized vertically according to which PC is being investigated (1, 2, 3, 4) and horizontally according to the level of filtering that was applied prior to running PCA (*none*: all SNPs, *exclude*: after excluding regions in Table 1, *prune*: after LD pruning with an r^2 threshold of 0.1 and window size of 0.5 Mb, and *both*: after both exclusions and LD pruning).

it remains to be fully understood what the downstream implications would be of adjusting for these PCs in genome-wide association studies. We conducted a simulation study to investigate these implications further.

Figure 6 presents Manhattan plots from one replicate of our simulation study. In this setting, there is a single causal variant on chromosome 4, and we compare the results from genome-wide association studies in WHI SHARe African Americans using different ancestral heterogeneity adjustment approaches. As expected, we see extreme inflation, i.e., statistically significant associations on *every* chromosome, when we do not make any adjustment for ancestral heterogeneity (Panel A). Otherwise, when we infer and adjust for ancestral heterogeneity using either PCA or a model-based approach, we see a single peak in our Manhattan plot on chromosome 4—as hoped, given that is where the causal variant is located—with one notable exception. When we adjust for the first four principal components (as has been done in previous GWAS in WHI SHARe^{32,58}), where those PCs were generated without any prior LD-based pruning or filtering, then we see a spurious association on chromosome 6 (Panel C). However, this spurious association disappears if we only adjust for the first of these PCs (Panel B). Likewise, no spurious association arises if we adjust for model-based admixture proportions (Panel D) or if we use PCs that were generated after LD pruning and Table 1 exclusions (Panels E and F). Note that the causal variant, on chromosome 4, and the spurious signal, on chromosome 6, are both located in regions of the genome that are highly correlated with the PCs that were generated without any prior LD pruning (Figure 5).

These results are not unique to this simulation setting. Figure 7 presents a comparison of the rate of spurious associations in genome-wide association studies in WHI SHARe African Americans. We see that, across all simulation settings, adjusting for PCs that capture local genomic features leads to higher numbers of spurious associations. Comparing models that make some sort of adjustment for ancestral heterogeneity, we observe the most spurious associations when GWAS models adjust for four principal components that were generated

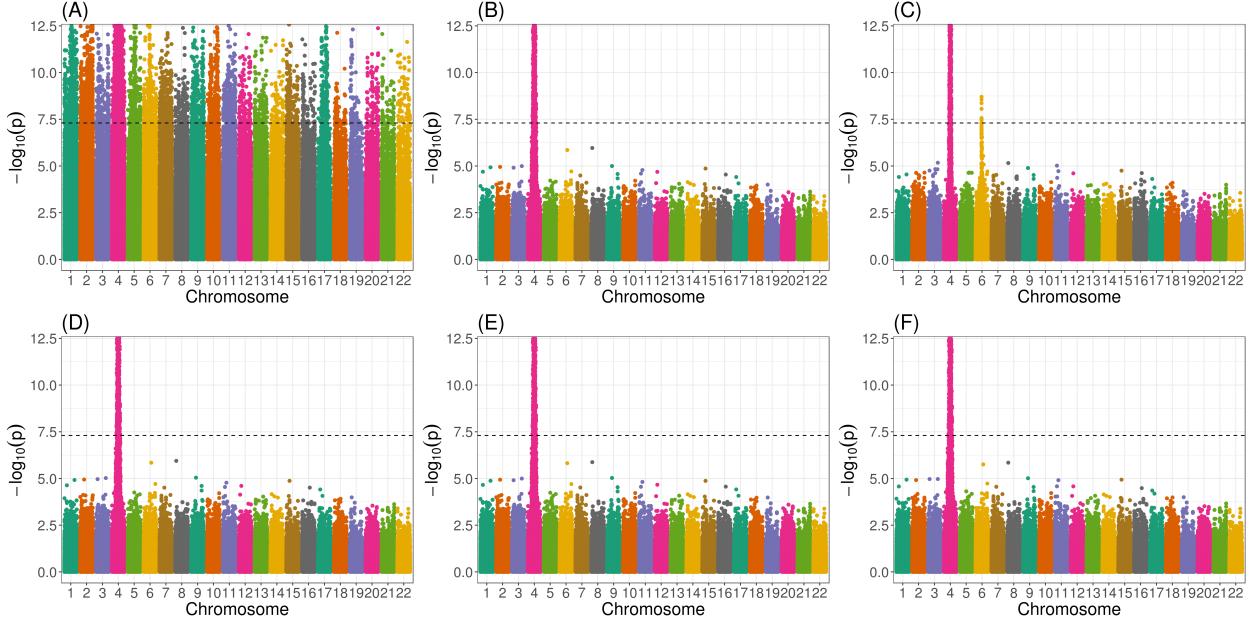


Figure 6: Manhattan plots from genome-wide association studies in WHI SHARe African Americans using different approaches to adjust for ancestral heterogeneity. In this example, the simulated trait depends only on the genotype at a single variant on chromosome 4: $y \sim N(g_{rs2036153}, 1)$. Panels present results using different adjustment approaches: (A) no adjustment; (B) one PC, with PCs calculated using all variants; (C) four PCs, with PCs calculated using all variants; (D) model-based admixture proportion estimates; (E) one PC, with PCs calculated after LD pruning ($r^2 < 0.1$, window size = 0.5 Mb) and Table 1 exclusions; and (F) four PCs, with PCs calculated after LD pruning and exclusions.

without any LD-based pruning or exclusions. Excluding the high LD regions from Table 1 prior to running PCA reduces the number of observed spurious associations slightly, but not to the levels of the other approaches. Given what we saw in Figure 5, this is perhaps not surprising: even with these exclusions, PCs 2–4 still capture local genomic features—unless those exclusions are also combined with strict LD pruning. We see far fewer spurious associations when models adjust for model-based admixture proportions or principal components that do not capture local genomic features (i.e., using just the first PC, regardless of LD-based pruning or exclusions, or adjusting for four PCs when LD pruning was performed).

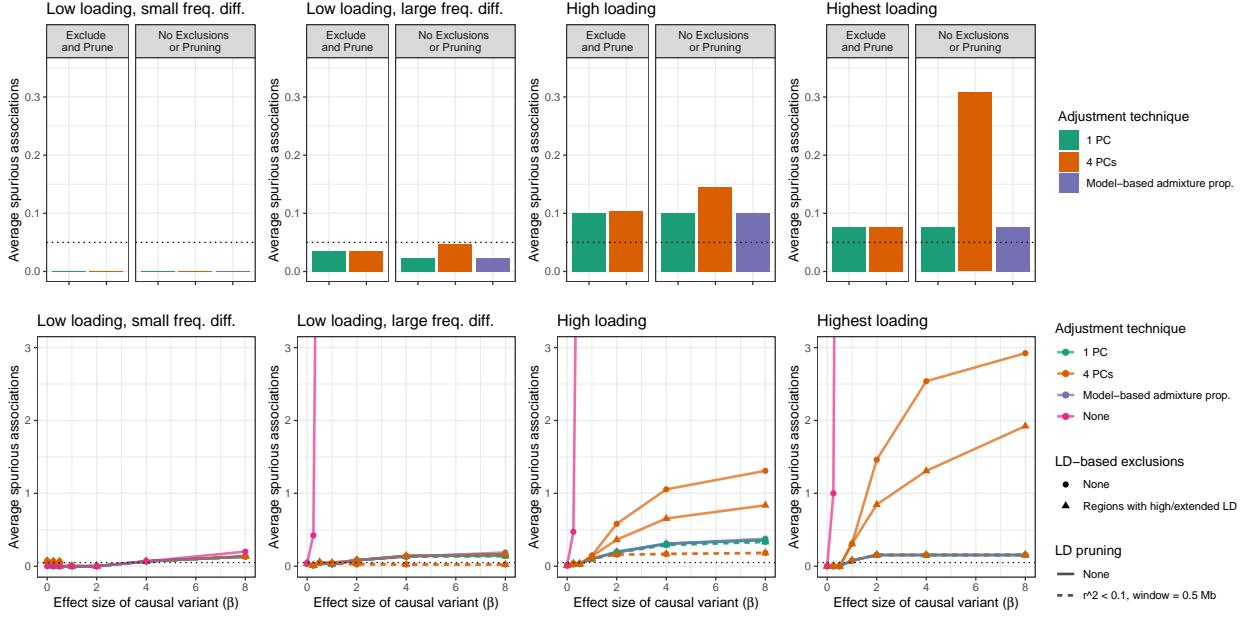


Figure 7: Comparison of the average number of spurious associations in genome-wide association studies in WHI SHARe African Americans. FLIP top and bottom, add all, remove "low loading" from first panel title, remove "large freq diff" from second panel title; make two versions: one with beta = 1 and the other with all betas and see what collaborators think [... DESCRIBE MORE ...]

3.6 Factors that influence when spurious associations will arise

Our simulations also highlight a number of factors that influence the number of spurious associations that arise when adjusting for PCs that capture local genomic features. In Figure 6, we saw that a spurious association arose when we adjusted for PCs that were highly correlated with variants in several regions across the genome, and both the causal variant and spurious signal were located in one of those regions. Again, this pattern is confirmed by the other replicates of our simulation study. [... What factors influence rate of spurious assoc?

- Difference in allele frequencies at causal variant (then all approaches have small number of spurious assoc, even no adjustment — no confounding, so really no need to adjust here)
- Is causal variant correlated with PC? (the larger the correlation between the causal

SNP and the PCs, the worse the problem)

- Effect size of causal variant

...]

[... Note to add in somewhere: unsurprisingly, given the high correlation we observed between model-based admixture proportions and PC1 (with or without LD pruning), we see very similar results for these three adjustment approaches ...]

[... why do spurious assoc arise?

- discuss theoretical results, connect to idea of collider bias

...]

4 Discussion

Need to address ancestral heterogeneity in admixed populations

- we observe considerable heterogeneity in global ancestry proportions in admixed populations studied here, as in other studies
- well-established that global ancestry is a potential confounding variable
- this confounding can exist even if global ancestry does not have a direct effect on the trait (as demonstrated by our simulation studies and theoretical results)
- → important to carefully measure and adjust for ancestral heterogeneity in GWAS in admixed populations

Comparing (naive) PCs and admixture proportions

- both widely used for measuring and adjusting for ancestral heterogeneity
- in AA, first PC correlated with global ancestry but later PCs are not (in HL: TBD)

- instead, later PCs often capture local genomic features (e.g., regions with extensive LD)
- while this has been documented before, note that, in contrast to what has been observed in EUR, we see that PCs seem to capture SNPs on more than one chromosome (multiple peaks in SNP loading plots); whereas in EUR we often just see one peak (cite Zou, Prive, other examples?) → why? LD patterns in admixed pops differ from those in EUR

[... for discussion:

- why is this happening? (LD)
- how does what we see compare to what's been observed in Europeans?
- does LD pruning universally fix the problem (i.e., does it seem to work better in some samples than others)? how many PCs does it help (i.e., what do PCs 5–10 look like)?
- why do we think exclusions didn't work? (high LD regions identified in Europeans, patterns of LD differ—more extensive—in admixed populations)

...]

Spurious associations

- adjusting for these PCs can lead to spurious associations
- this is due to a phenomenon known as collider bias
- ADD: what do theory and sims tell us about when/how likely a spurious association is to occur?
- ADD: could spurious associations replicate? (given that peaks often occur in similar places across datasets)

Impact of LD pruning and removing high LD regions

- after LD pruning, PCs no longer exhibit patterns of being driven by select few SNPs (at least for PCs 2-4)
- note that we had to use smaller r^2 and wider windows than often recommended in literature for this to be true → why? LD patterns in admixed pops differ from those in EUR
- excluding previously-identified high LD regions doesn't seem to be as effective → why? LD patterns in admixed pops differ from those in EUR
- note, too, that even strict LD pruning doesn't seem to remove all correlation between SNPs and genotypes (e.g., later PCs in WHI, TOPMed COPDGene)

Recommendations

- If using PCs, carefully inspect SNP loadings and/or correlation between PCs and genotypes
- If using PCs, don't use more than you need
- Consider using global ancestry proportions (although further work is needed to reliably capture sub-continental structure)

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

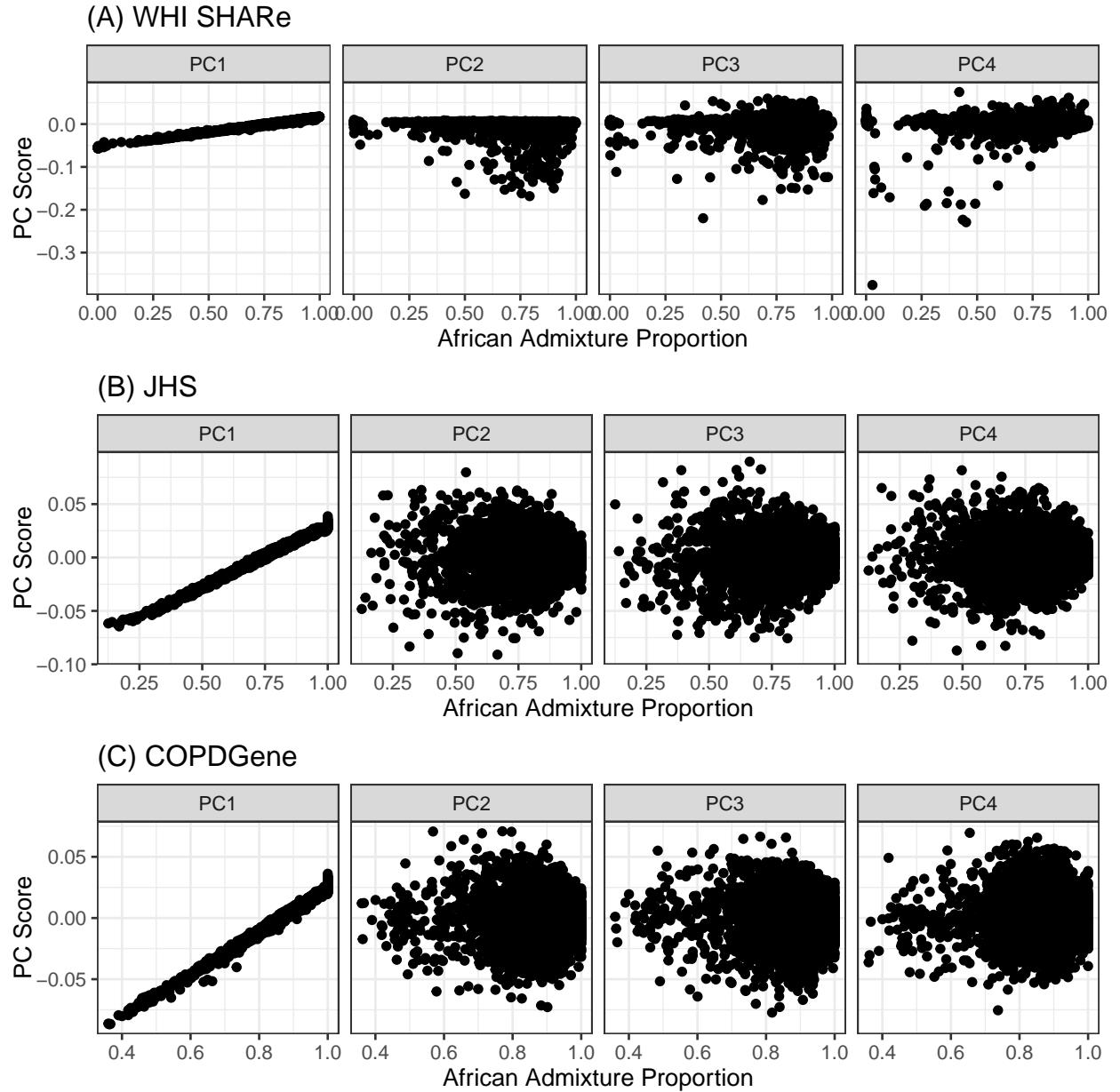


Figure 8: Scatterplots of estimated African admixture proportions versus the first four PCs in WHI SHARe (Panel A), TOPMed JHS (Panel B), and TOPMed COPDGene (Panel C) African Americans. Here we consider PCs that were generated after LD pruning ($r^2 = 0.1$, window size = 0.5 Mb) and filtering previously identified high-LD regions (1).

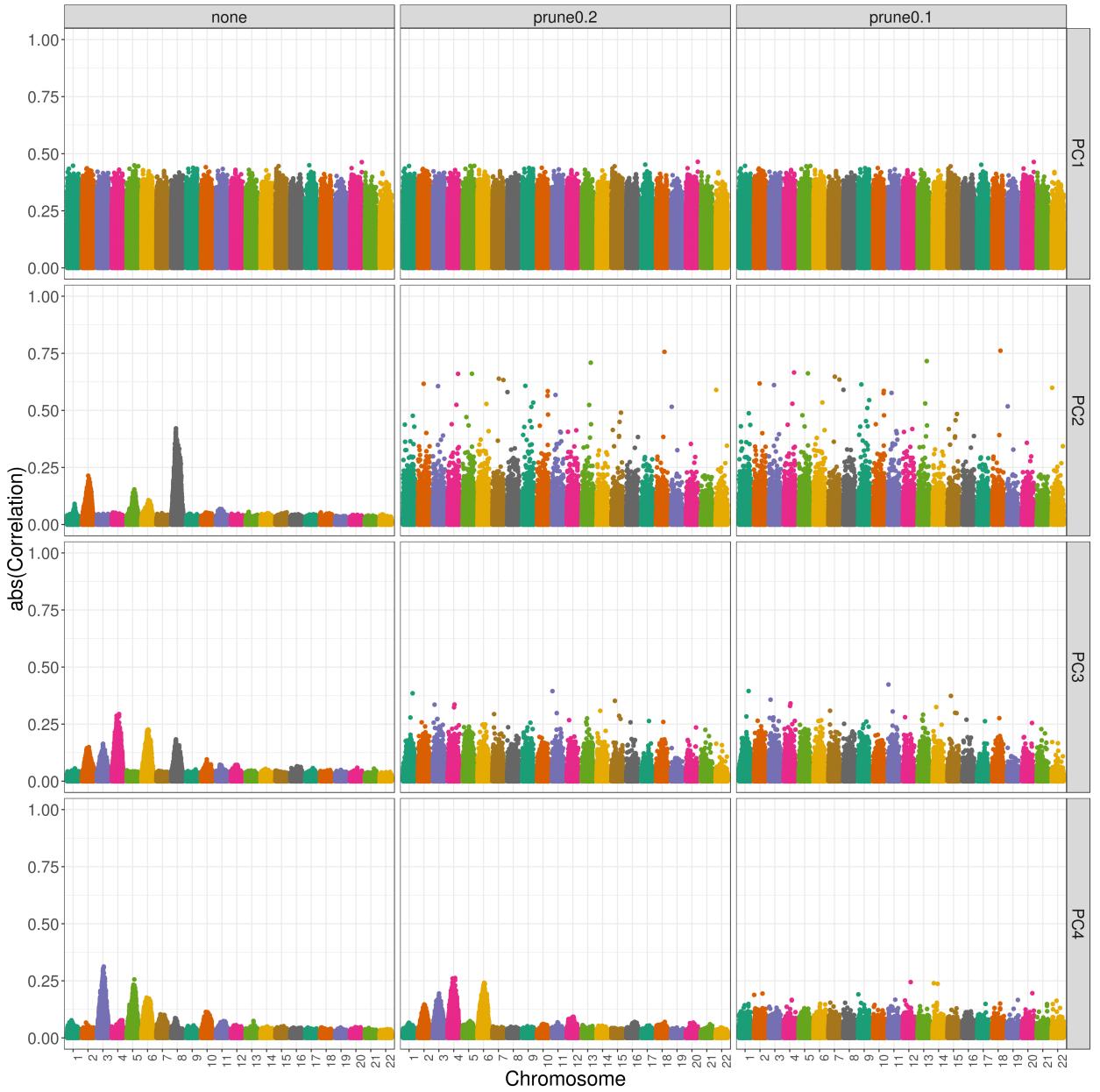


Figure 9: Correlation between PCs and genotypes in WHI SHARe African Americans using different LD pruning thresholds. Each panel plots the absolute value of the correlation between principal components and genotypes (on the y-axis) versus the position along the genome (x-axis). Panels are organized vertically according to which PC is being investigated (1, 2, 3, 4) and horizontally according to what r^2 threshold was used when running LD pruning prior to PCA (*none*: no LD pruning, *prune0.2*: LD pruning with an r^2 threshold of 0.2 and window size of 0.5 Mb, and *prune0.1*: LD pruning with an r^2 threshold of 0.1 and window size of 0.5 Mb).

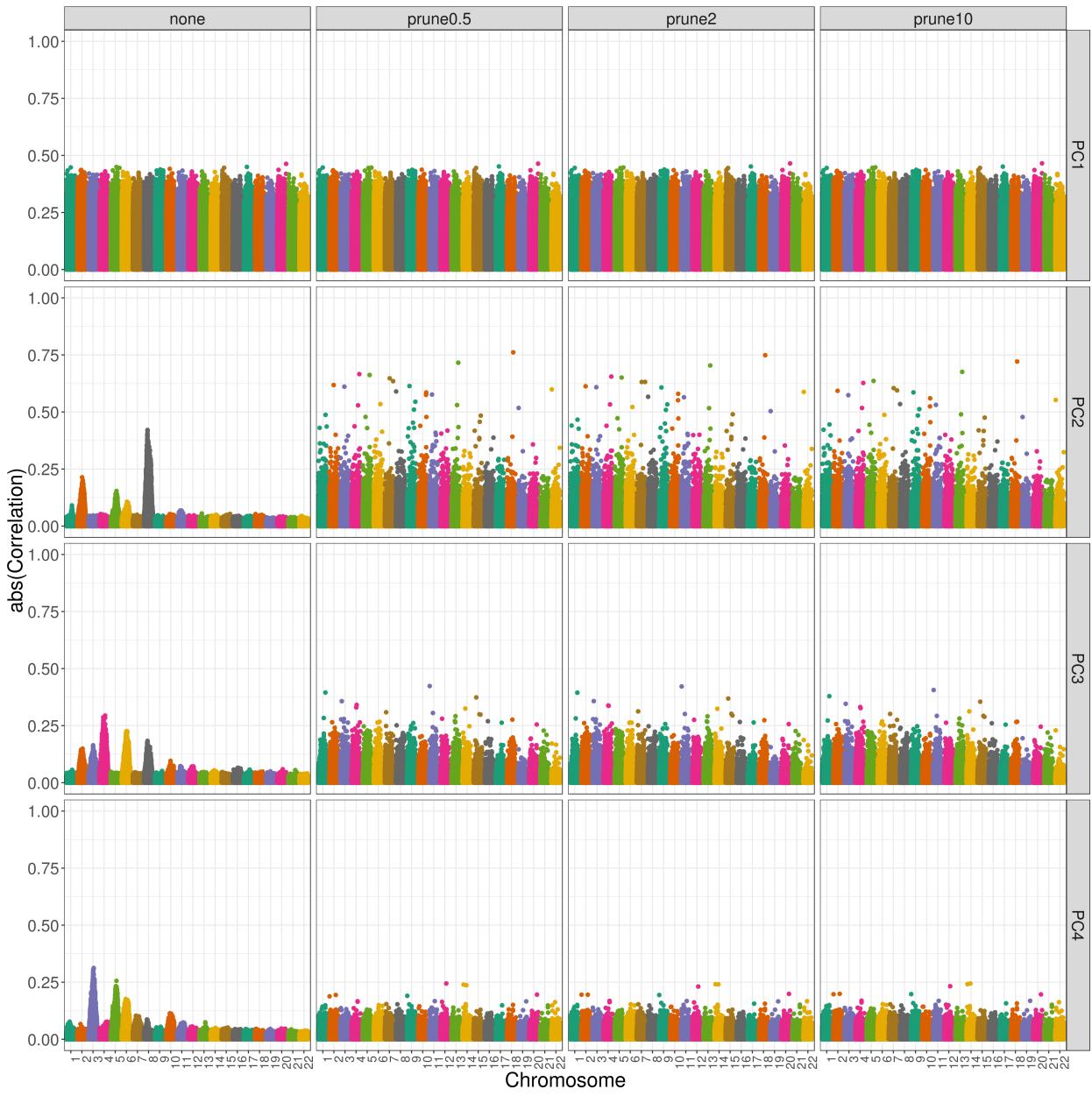


Figure 10: Correlation between PCs and genotypes in WHI SHARe African Americans using different LD pruning window sizes. Each panel plots the absolute value of the correlation between principal components and genotypes (on the y-axis) versus the position along the genome (x-axis). Panels are organized vertically according to which PC is being investigated (1, 2, 3, 4) and horizontally according to what window size was used when running LD pruning prior to PCA (*none*: no LD pruning, *prune0.5*: LD pruning with an r^2 threshold of 0.1 and window size of 0.5 Mb, *prune2*: LD pruning with an r^2 threshold of 0.1 and window size of 2 Mb, and *prune10*: LD pruning with an r^2 threshold of 0.1 and window size of 10 Mb).

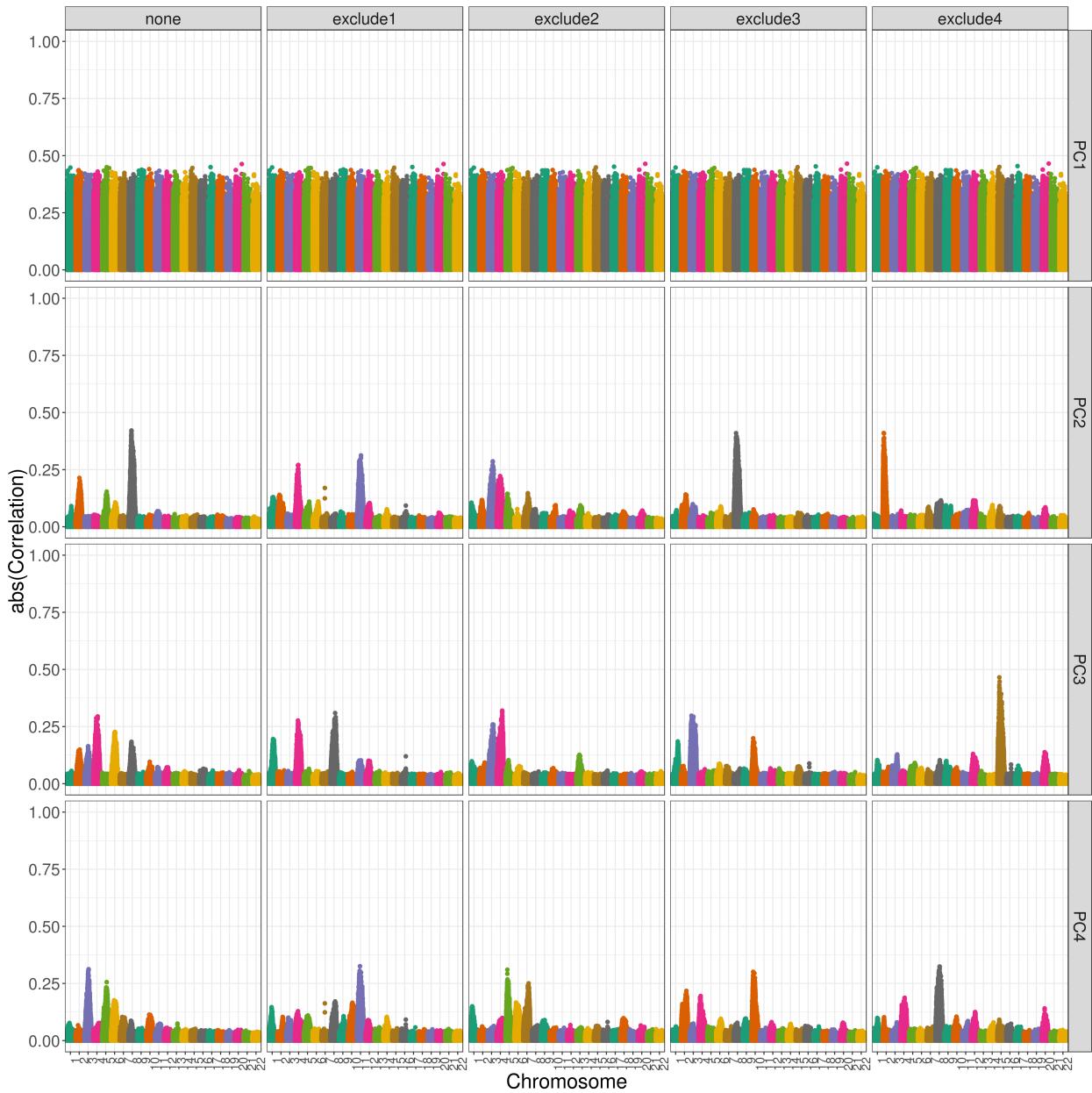


Figure 11: Correlation between PCs and genotypes in WHI SHARe African Americans after multiple rounds of data-based exclusions. Each panel plots the absolute value of the correlation between principal components and genotypes (on the y-axis) versus the position along the genome (x-axis). Panels are organized vertically according to which PC is being investigated (1, 2, 3, 4) and horizontally according to the number of iterations of our procedure for excluding regions highly correlated with PCs that were implemented prior to PCA (*none*: no exclusions, *exclude1*: one round of exclusions, *exclude2*: two rounds of exclusions, etc.).

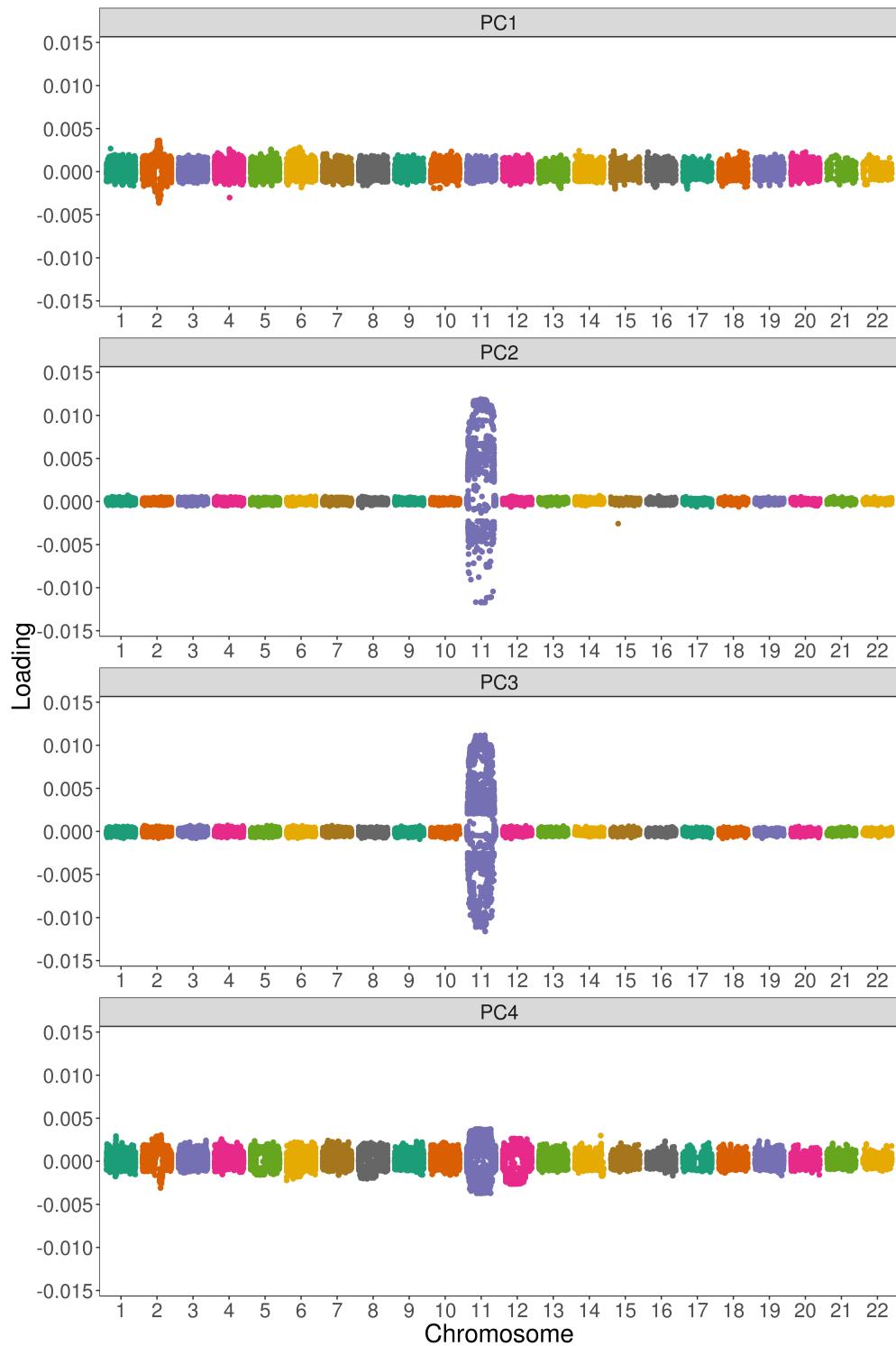


Figure 12: SNP loadings for naively generated PCs in COPDGene European Americans. Each panel plots the principal component loading (y-axis) versus the position along the genome (x-axis) for each variant. Panels are organized vertically according to which PC is being investigated (1, 2, 3, 4).

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Figure Titles and Legends

Tables