A genetic similarity measure for identifying fine-scale population stratification and cryptic relatedness

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Abstract

The improving quality and falling costs of next-generation sequencing has allowed for vast increases in availability of these data. With this increased abundance comes a new ability to investigate population structure with previously unattainable precision. In addition to the anthroplogical value, properly accounting for confounding in GWAS, particularly for rare variants, is of meaningful importance. [More about rare variants, recent migration, relatedness, etc...]

Introduction

The impact of confounding due to population structure and cryptic relatedness is well established [10–12]. Many approaches are utilized to address this concept at the analysis level, such as PCA [8, 9], mixed models [3, 5, 15, 16], etc. or at the data collection level by limiting the scope of the study to homogeneous populations or matching by ancestry. However, as our ability to further detect structure increases with developing methods and technologies we find that even supposedly homogeneous populations contain detectable population structure that may inflate type I error particularly among rare variants [6]. Additionally, the presence on cryptic relatedness presents further potential for confounding in GWAS, increasing both type I and type II errors [4, 13, 14].

Much of the structure identifcation improvement can be attributed to our ability to identify rare alleles. Rare alleles may be exploited due to the fact that they are more likely to have arisen recently and thus a superior approach towards separating populations that have recently separated [7]. Furthermore,

very low allele frequencies are more unstable than higher allele frequency, either becoming fixed at zero or becoming more common with high probability.

In this study we present a genetic similarity measure that is designed to separate individuals with recent common ancestry. Our measure has clearly defined properties which can be used to test for homogeneity in a population and in particular identify individuals who are likely be related in a study population.

Methods

Exploiting the relative value of rare alleles is fundamental to our method, which uses an intuitive, computationally straightforward approach towards identfying similarity between two individuals. Effectively, we give a larger weight to a genotype which is common to two individuals if the allele frequency is low among the rest of the population.

For a matrix of n individuals ($2n$ haploid genomes), with N variants described by the genotype matrix $\mathbf{G}_{2n\times N}$, we define the weighted Jaccard similarity between two haploid genomes, $s_{i,j}$

$$s_{i,j} = \frac{\sum_{k=1}^{N} w_k \mathbf{G}_{i,k}\mathbf{G}_{j,k}}{\sum_{k=1}^{N} I\left[\sum_{l=1}^{2n} \mathbf{G}_{l,k} > 1\right]}$$

where

$$w_k = \begin{cases} \frac{\left(\sum_{l=1}^{2n} \mathbf{G}_{l,k}\right)}{\binom{2n}{2}} & \sum_{l=1}^{2n} \mathbf{G}_{l,k} > 1 \\ 0 & \sum_{l=1}^{2n} \mathbf{G}_{l,k} \leq 1 \end{cases}$$

$$E\left(s_{i,j}|\text{No structure}\right) = 1$$

It therefore follows from the central limit theroem that in the absence of populations structure, cryptic relatedness and dependence between loci (such as linkage disequilibrium) the distribution of the test statistic, $s_{i,j}$ is Gaussian.

$$s_{i,j} \sim N\left(1, \sigma^2\right)$$

Where σ^2 is estimated by

$$\hat{\sigma^2} = \hat{Var}\left(s_{i,j}|\text{No structure}\right) = \frac{\sum_{k=1}^{N} \hat{p}_k^2\left(1-\hat{p}_k^2\right) w_{k,i,j}^2}{\left(\sum_{k=1}^{N} I\left[\sum_{l=1}^{2n} \mathbf{G}_{l,k} > 1\right]\right)^2}$$

This provides an easily interpreted statistical test for evaluating possible relatedness between individuals in a purportedly homogeous dataset of unrelated individuals.

Furthermore, this measure is particularly sensitive for measuring relatedness. Intuitively, we can imagine two subjects which have a kinship coefficient, Φ,

indicating a probability of a randomly chosen allele in each person being identical by descent (IBD). For an allele which belongs to the one person, the probability of it belonging to the related person is $\Phi + (1 - \Phi) \times p$, where p is the allele frequency in the population. We can clearly see that for rare alleles, such that p is small compared to Φ, there will be a much larger relative difference in the probability of shared alleles among related individuals ($\Phi > 0$) compared to unrelated individuals ($\Phi = 0$). Given that our method weights more highly these rarer alleles, there is increased sensitivity to detection of relatedness.

Consider a coefficient of relatedness, $\Phi > 0$,

$$\begin{aligned} E\left(s_{i,j} | \Phi, \text{No other structure}\right) &= \sum_{k=1}^{N} w_k \left(\Phi p_k + (1 - \Phi) p_k^2\right) \\ &> 1 \end{aligned}$$

For example, in an otherwise homogeneous population of unrelated individuals an uncle-nephew relationship ($\Phi = .125$), with $MAF \sim Uniform\left(.01, .1\right)$ we can directly calculate the expectation of their similarity statistic, $s_{i,j}$

$$E\left(s_{i,j} | \Phi = .125, \text{No other structure}\right) \approx 2.9$$

This approach is easily generalized to the diploid scenario. A diploid similarity score, $s_{diploid}$, is obtained by averaging each of the four pairwise haploid $s_{haploid}$ scores between each person's two haploid genotypes. For N individuals, $2N$ genotypes per loci, the similarity between individuals i and j is defined as

$$s_{diploid,i,j} = \frac{\sum_{k=1}^{N} \left[w_k \mathbf{G}_{i_1,k} \mathbf{G}_{j_1,k} + w_k \mathbf{G}_{i_1,k} \mathbf{G}_{j_2,k} + w_k \mathbf{G}_{i_2,k} \mathbf{G}_{j_1,k} + w_k \mathbf{G}_{i_2,k} \mathbf{G}_{j_2,k}\right] / 4}{\sum_{k=1}^{2N} I\left[\sum_{l=1}^{2n} \mathbf{G}_{l,k} > 1\right]}$$

where $\mathbf{G}_{i_2,k}$ refers to the 2^{nd} genotype of individual i at locus k.

This formulation will have the same mean

$$E\left[s_{diploid,i,j}\right] = 1$$

and assuming independence of each individual's haploid genomes, such as in the absence of inbreeding,

$$\hat{Var}\left(s_{diploid,i,j} | \text{No structure}\right) = \frac{\hat{Var}\left(s_{haploid,i,j} | \text{No structure}\right)}{4}$$

Identification of relatedness in 1000GP data

We applied our method to data from the 1000 Genomes Project [1,2], a consortium...[].

These populations were not identified to have cryptic relatedness or had cryptic relatedness removed **[citation difficult (pptx file posted online KGP**

website) ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/phase1/analysis_results/supporting/cryptic_relation_analysis/Nemesh_crypticrelatedness_20120213.pptx].

Phase 3 of the 1000 Genomes Project contains approximately 2504 individuals with a combined total of 88 million variants. To test our method, we sampled 80,000 variants uniformly spaced in the dataset in order to limit the impact of linkage disequilibrium. Our method was then run on each of the 26 populations in the study as well as on 5 super populations and the study as a whole.

We discovered that there was great variation in the presence of cryptic relatedness and population structure across the 26 populations of the study. Under the assumptions that each study contained a homogeneous population of unrelated individuals, only a handful of groups contained neither large outliers nor heavily inflated numbers of significant results.

We defined the presence of population structure as applying to those populations which had a median $s_{i,j} < .97$. Using this criterion, three of the 26 populations met this threshold- MXL (Mexican Ancestry from Los Angeles USA), PUR (Puerto Ricans from Puerto Rico), and PEL (Peruvians from Lima, Peru). Each of these populations are "new world" populations which have undergone extensive admixture in the past centuries. It is therefore unsurprising that these groups of individuals would exhibit the greatest amount of heterogeneity among the populations surveyed.

We defined the presence of cryptic relatedness as those individual pairs which exceed the cutoff for a family-wise error rate of $\alpha = .01$. Cryptic relatedness was discovered in 12 **[update this with latest results]** of the 26 populations using this method.

The overlap in these two groups may be due to the fact that the variance in similarity is inflated in the presence of population structure. So it is not accurate to identify cryptic relatedness in this manner in popultations which contain structure. However, in populations which do not exhibit detectable structure, we still find many instances of related individuals in this study. For example, two individuals from the ACB population (African Caribbeans in Barbados) had a $s_{i,j}$ score of 2.6 $\left(p < 10^{-30}\right)$, whereas no other pairing exceeded the family-wise cutoff of 1.3 ($p = .0002$). Using the formula above, we estimate this relationship to be $\Phi = X$ [Redo this analysis], indicating...

Population detection in 1000 Genomes Project

There are many methods for detecting population structure. Most commonly, Principal Components Analysis [8, 9]is applied for identifying the components of largest variation which ideally corresponds to the population structure. This procedure first involves the calculation of a genetic similarity matrix (GSM) via the correlation between all samples, which is commonly followed by an eigendecomposition of that matrix. There are a number of limitations to this straightforward approach, one of which is that the calculation of a variance-covariance matrix equally weights the impact of all loci **[unless standardized by rows?]**,

Distribution of similarity statistic within population subgroups from 1000 Genomes Project

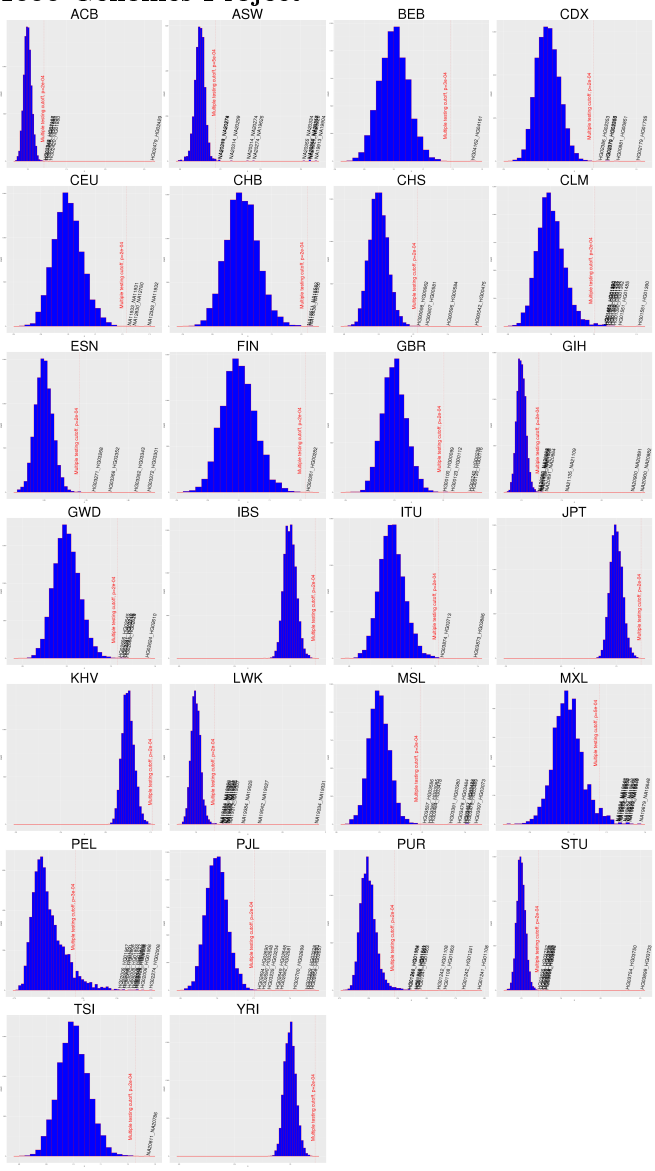


Figure 1: Distribution of similarity coefficients for each of the 26 populations in the 1000 Genomes Project. Homogeneous populations lacking cryptic relatedness should be expected to exhibit distributions centered around 1 with no outliers. The red dotted vertical line on each plot indicates the family-wise$\alpha = .05$ level cutoff for $\binom{n}{2}$ comparisons. Many of the population groups do demonstrate the null behavior (e.g. JPT, KHV, FIN)- however, a number of populations show the presence of extreme outliers (e.g. STU, PUR) or systematic right skew (e.g. MXL, PEL)

Population	Structure	Cryptic Relatedness
ACB	No	Yes
ASW	No	Yes
BEB		
CDX	No	Maybe
CEU	No	No
CHB	No	No
CHS	No	Yes
CLM	No	No
ESN	No	Yes
FIN	No	No
GBR	No	No
GIH	No	Yes
GWD	No	No
IBS	No	No
ITU	No	Yes
JPT	No	No
KHV	No	No
LWK	No	Yes
MSL	No	Yes
MXL	Yes	Yes
PEL	Yes	No
PJL	No	Yes
PUR	Yes	Yes
STU	No	Yes
TSI	No	No
YRI		

Table 1: Presence of population structure and cryptic relatedness detected in each of the 26 populations in the 1000 Genomes Project. Population structure, defined as a $median\,(s) < .97$ was found in three groups- MXL, PEL and PUR. Each of these populations are "new world" populations which have undergone extensive admixture in the past centuries.

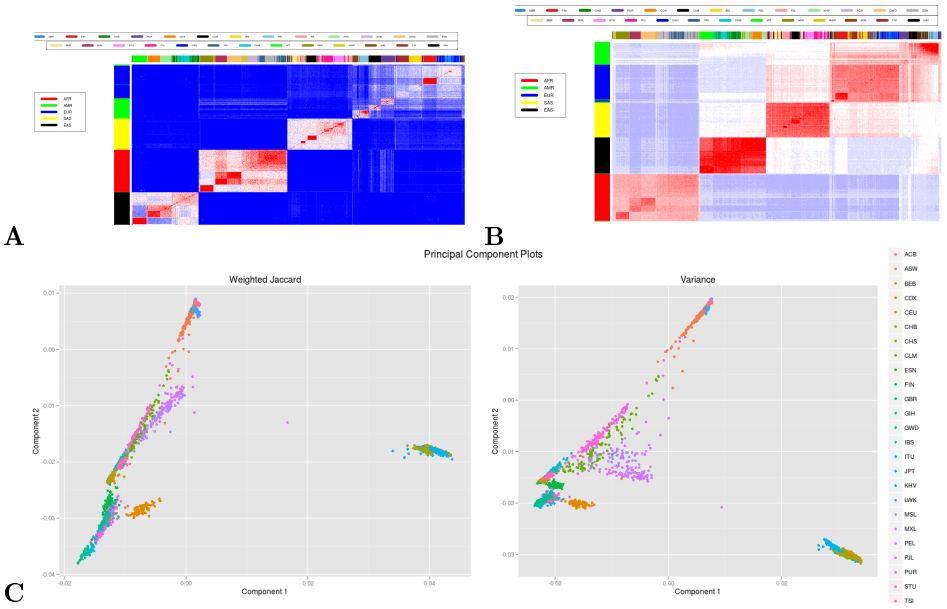


Figure 2: Heatmap of GSM generated by our method (A) and variance-covariance (B) using 80,000 uniformly spaced variants. Samples have been ordered by hierarchical clustering (dendrogram no shown). The vertical colorbar indicates membership in one of the five superpopulations, while the horizontal colorbar indicates membership in one of the 26 populations. (C) Projecting each individual onto the top two eigenvectors resulted in a similar 2-dimensional distribution of global ancestry

failing to fully utilize the fact that the overall allele frequency is informative of the value of each variant.

We used the 1000 Genomes Project data to compare the GSMs obtained via the conventional variance-covariance matrix step and the use of our method. We evaluated the ability of each method to separate the same quantity of data into the 5 superpopulations and 26 populations. Using approximately 80,000 **[Adjust for filtered]** variants, we generated the two GSMs and plotted the similarity matrices, ordered by hierarchical clustering with average linkage (Figure 3).

Both methods performed well at separating the five superpopulations, but the our method outperformed variance covariance in separating populations of the same superpopulation. As expected, the lack of focus on less frequent alleles, which are more important for distinguishing recent ancestry allowed variance-covariance to adequately separate continental origins, but failed to sufficiently partition the samples according to subgroups.

The first two eigenvectors of the GSMs generated using our method vs variance-covariance yield very similar results. Both methods provide a suffi-

cient separation of coarse-scale population structure. But closer examination of fine-scale population structure reveals our method to be an improvement over variance-covariance. We were able to provide a stronger separation of all 26 populations, particularly those of recent ancestry. As an example, we explored two populations- Sri Lankan Tamil and Indian Telugu, which have relatively small geographical separation.

Interestingly, we found a strong case for cryptic relatedness between three pairs of individuals, one pair of which (HG03998 and HG03873) spanned the two populations. Considering that both groups were sampled in the UK, this suggests a distant genetic relationship is possible from members of different population groups.

Rarer alleles are more informative for recent ancestry

More figure and text, not sure where this goes

Separation of recent shared ancestries

Example: Indian Telugu from the UK (ITU) Sri Lankan Tamil from the UK (STU)

Separation of recent shared ancestries

Example: Iberian Population in Spain (IBS) Toscani in Italia (TSI)

Separation of recent shared ancestries

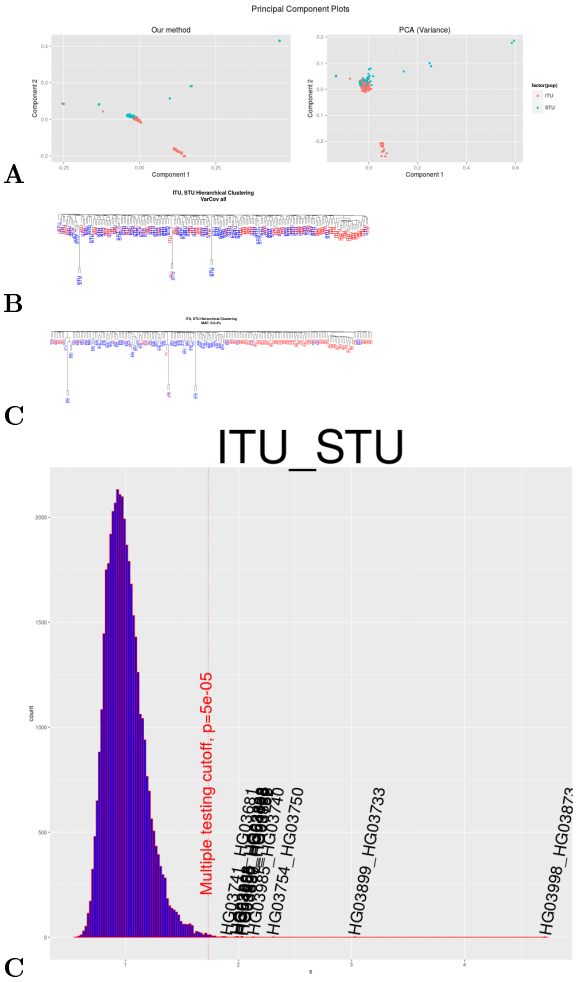
Ratio of within-group mean distance to out-of group mean distance:

Populations	Our method	PCA
TSI-IBS	.417	.504
BEB-PJL	.748	.794
ITU-STU	.836	.889
ITU-BEB	.905	.951
CHB-CHS	.605	.681
LWK-ESN	.178	.197
GIH-ITU	.513	.552
CEU-YRI	.025	.022

Our method outperformed standard PCA in differentiating groups for *every* same-continent subpopulation pairing across all continents. (≈ 50 comparisons)

dddd

The Mathieson paper (Nature Genetics, 2012) proposed a problem wherein the case of sharply spatially defined phenotypic risk lead to the inflation of association test statistics for rare variants. Moreover, this effect was not resolved using existing methods for controlling for confounding by population stratification. The Listgarten response (Listgarten et al, Nature Genetics 2012) claimed to solve this with their novel method LMM-select. This method effectively



[Rerun this figure on whole dataset]

Figure 3: **Example: ITU vs STU**. Two populations of Southern Asian origin, Indian Telugu from the UK (ITU) and Sri Lankan Tamil from the UK (STU) show poor separation using the variance-covariance approach. When using our method, we see improved separation of populations when individuals are projected onto the top two eigenvectors (**A**) despite the fact that our method appears to have expended a greater proportion of the variance explanation on identification of related individuals. Hierarchical clustering using the GSM as a similarity matrix (**B**) was unable to clearly visually distinguish between ITU and STU, but use of our method provided much clearer results (**C**).

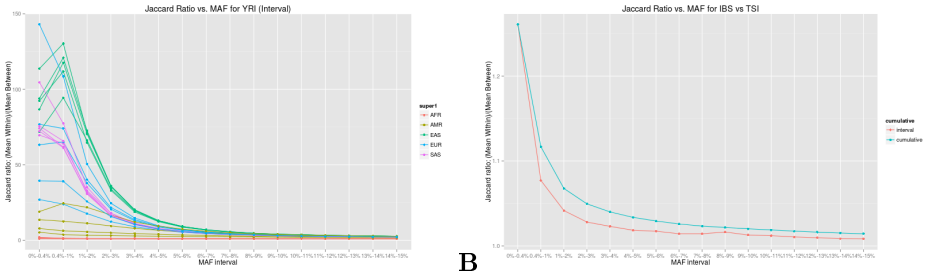


Figure 4: (**A**) Ratio for mean within-population jaccard ratio vs mean out-of-population jaccard ratio for Yoruba in Ibadan, Nigeria (YRI) compared to all other populations. We find that in comparising YRI to all other populations, the within-population vs out-of-population ratio increases as the allele frequency decreases. This trend held true for all 26 populations. For the smallest allele frequency bin, 0%-0.4%, the trend is not as clear suggesting that this is the point at which quality control is of concern when considering rare variants. (**B**) Ratio for mean within-population jaccard ratio vs mean out-of-population jaccard ratio for two populations with a relatively recent common ancestry- Iberian Population in Spain (IBS) and Toscani in Italia (TSI). Although the ratio is unsurprisingly on a smaller scale, we find that the most informative variants are those with the smallest allele frequency.

uses a linear mixed model approach but rather than use all variants to generate their genetic similarity matrix, selects only those which are correlated with phenotype. A more recent paper on which Listgarten was an author (Widmer, Further Improvements..., Nature 2014), appears to reverse this claim. In this paper, Widmer admits one potential improvement, building a GSM based on selected SNPs that well predict the phenotype failed rather dramatically. In particular, when population structure, family relatedness, or both were present, this approach failed to control for type I error. Seemingly indicating that their group's previous claim was premature. However, they claim now that inclusion of principal components as fixed effects does properly control for type I error. [However, I don't see it demonstrated in the paper and I remain somewhat unclear skeptical about what definition they are using]. (For example, we are focusing on extreme events, where inflation is only measurable at $\approx 10^{-6}$)

A possible explanations for the test statistic inflation was suspected to be primarily due to lack of a precise measure of genetic similarity. This was attributed in part due to the fact that linear methods such as PCA would have difficulty expressing sharply defined risk regions.

However, we found that test statistic inflation persisted even when controlling for the exact regions for which we simulated differential phenotypic risk. This demonstrated that although proper estimation of the genetic similarity matrix is necessary, it is not sufficient to control type I error. As we show here, association tests in the presence of properly controlled population stratification

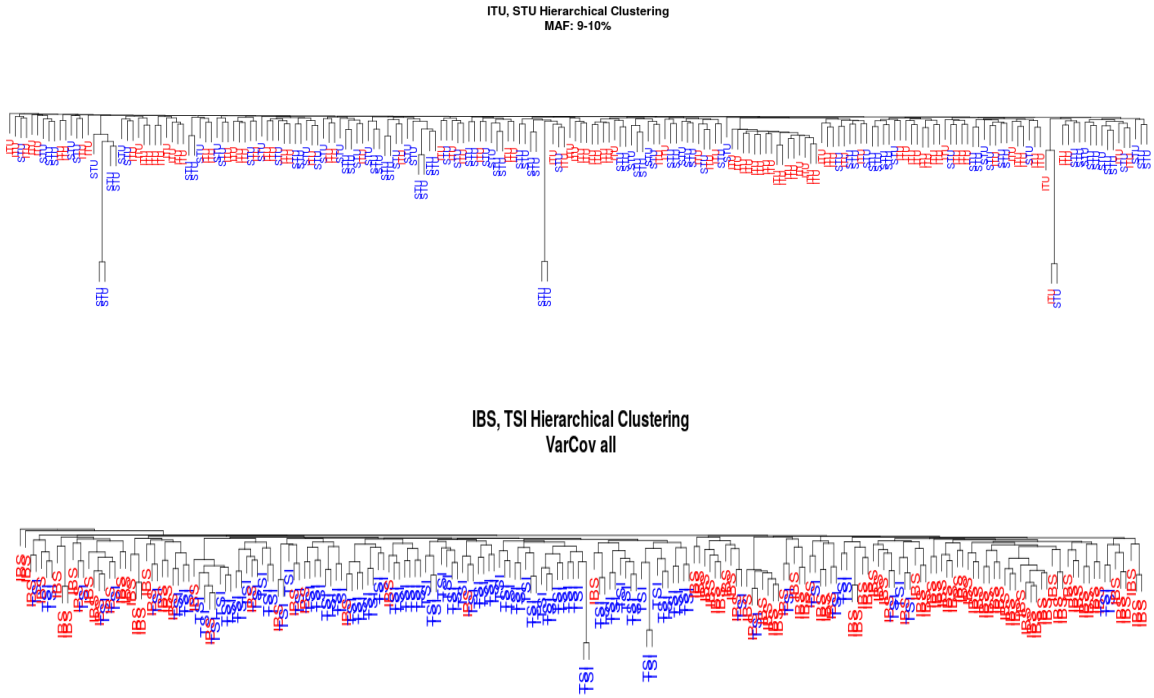


Figure 5: Figures...

exhibit overdispersion due to the existence of high leverage individuals after adjusting for confounding.

A consistent estimator of rare-allele association in stratified GWAS We developed a statistic which is asymptotically unbiased by calculating the variance of the observed squared correlation statistic in the presence of differential variance for both spatially-adjusted genotypes and spatially-adjusted phenotypes. Intuitively, with uniform variance across samples (such as with no population stratification) we expect the variance of the correlation to be asymptotically $\sqrt{\frac{1}{n}}$. In this case, we have the Armitage Trend Test statistic- $n \times r^2 \sim \chi^2_1$. However, in the presence of adjusted population stratification the variance of the observed correlation is not $\sqrt{\frac{1}{n}}$. Depending on whether the correlation of the variances of adjusted genotypes and adjusted phenotypes are positive or negative we see that the variance of the observed correlation is greater than or less than sqrt(1/n), respectively. Insert derivation here

In combination with our estimated test statistic, we propose a method for estimating the GSM by using the Jaccard Similarity Index (JSI) for alleles which are present in less than 2% of the population. Our method is motivated by the plausible expectation that rare alleles are more likely to have emerged most recently in human ancestral history and thus may be superior differentiators for groups which have a more recent ancestral divergence. An eigendecomposition of the JSI-GSM can then be used to to identify linear predictors of ancestry in a manner similar to Principal Components Analysis.

Jaccard Index vs Variance-Covariance approach. Use of Jaccard similarity for rare alleles only in estimating GSM outperformed the standard variance covariance approach.

Computation of the JSI for all pairs is computationally faster than the more commonly used variance-covariance method typically employed for PCA (maybe the same speed?)

Use of JSI provides clearer separation of closely related subpopulations The ability to separate distinct subpopulations with a recent shared ancestry, such as Spanish (IBS) and Italians (TSI), is a desirable goal in GWAS. Even with F_{ST} values smaller than .001, there is a clear bias when performing GWAS on non-genetic outcomes correlated with phenotype. The ability to separate closely related populations was found to be a function of the allele frequency interval.

Separation of Sri Lankan and Indian populations using the Jaccard Similarity and based on a global allele frequency of $<0.4\%$ (top) compared to a global allele frequency of ~15% (bottom)

The plot above shows the relative ability to separate two closely related populations based on the allele frequency used. We see a monotonically decreasing separation score as more common alleles are used indicating that the ancestry informativeness peaks when utilizing the rarest loci. We find that the estimated ratio of mean within-group JSI to between group JSI is maximized by excluding all but those alleles with $<.04\%$ MAF and that inclusion of higher frequency alleles reduces our ability to differentiate low Fst populations. Additionally, we find that although rare alleles may outperform more common alleles for closely

related populations the use of a higher cutoff for more distant populations may be appropriate. This could be attributed to a relatively greater sensitivity to quality control issues for groups which already have a clear genetic separation at higher allele frequencies. The plot below demonstrates the increased efficacy of less common alleles in separating Yoruban persons (YRI) from those populations from other continental origins. The Jaccard ratio drops at the lowest allele frequency interval, possibly due to the presence of randomly miscalled genotypes.

Simulations

We used real genotype data and simulated non-genetic phenotypes based on reported population membership in the 1000 Genomes Project. ...describe details of simulations...

estimator is consistent estimator is biased estimator is superior to rare-PCA -how to quantify?

Argue: is estimator superior to usual PCA? estimator is equivalent to subpopulation label?

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