Assessing the Impact of Population Stratification on Association Studies of Rare Variation

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Key Words

Rare variants · Population stratification · Genomic control · Principal component analysis

Abstract

Aims: The study of rare variants, which can potentially explain a great proportion of heritability, has emerged as an important topic in human gene mapping of complex diseases. Although several statistical methods have been developed to increase the power to detect disease-related rare variants, none of these methods address an important issue that often arises in genetic studies: false positives due to population stratification. Using simulations, we investigated the impact of population stratification on false-positive rates of rare-variant association tests. **Methods:** We simulated a series of case-control studies assuming various sample sizes and levels of population structure. Using such data, we examined the impact of population stratification on rare-variant collapsing and burden tests of rare variation. We further evaluated the ability of 2 existing methods (principal component analysis and genomic control) to correct for stratification in such rare-variant studies. **Results:** We found that population stratification can have a significant influence on studies of rare variants especially when the sample size is large and the population is severely stratified. Our results showed that principal component analysis performed quite

well in most situations, while genomic control often yielded conservative results. **Conclusions:** Our results imply that researchers need to carefully match cases and controls on ancestry in order to avoid false positives caused by population structure in studies of rare variants, particularly if genome-wide data are not available.

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Introduction

Genome-wide association studies (GWAS) have successfully identified 7,817 associations between common variants and 743 traits as of November 2012 (<http://www.genome.gov/gwastudies/>). However, the majority of these common SNPs have very small effect sizes (odds ratio between 1.1 and 1.5) and no apparent causal effects on the disease or trait of interest [1]. As GWAS are primarily designed to detect associations with common variants, an intuitive explanation of the missing heritability is that many common diseases are actually caused by rare variants [2, 3]. The published literature shows that the odds ratios of rare variants are often much larger than those of common variants [4]. Furthermore, rare variants are more likely to have causal effects than common variants in that they are expected to change amino acids and further influence interactions among proteins [4]. From an

evolutionary point of view, rare variants are rare either because they are selected against or because they are new and have not been under selection for a long time [5]. The above arguments suggest a role for rare variants in common disease and, with developments in cost-effective sequencing technology (which can obtain thousands of sequences in parallel), widespread search for rare susceptibility variants is now feasible. Sequencing studies have already identified several rare variants associated with common diseases including type 2 diabetes [6] and asthma [7].

Using the popular case-control design, many studies are attempting to identify rare causal variants that increase the risk for complex diseases. However, such studies require robust and powerful statistical tools for rare-variant analysis that are somewhat distinct from those used previously to analyze common variants. As the power to detect association with an individual variant is lower for less frequent variants, existing analytic methods typically used for GWAS are not powerful when applied to studies of rare variants. To avoid this power loss, most rare-variant methods collapse less frequent variants in a region together into a composite variable, and then test the association between the composite variable and the disease. For example, the Combined Multivariate and Collapsing (CMC) method by Li and Leal [8] collapses the rare variants in a region into a composite variable (according to predefined criteria) and then constructs a multi-marker test of association between the disease status and multiple composite variables (each composite variable corresponding to a different region). Another commonly used method is the burden test [9], in which the composite variable is calculated as the number of rare variants in a region. Simulation studies show that the burden test has higher power compared to other methods [9].

Although simulations suggested that these novel methods successfully increased the power to detect rare variants compared to standard tools used for the analysis of common variants, none of the available methods address an important issue that often arises in genetic studies: the potential for false positives due to population stratification. Population stratification is a systematic difference in allele frequencies between cases and controls caused by different subpopulation structures. It is well established that stratification, if not properly modeled, can lead to an increased number of spurious associations and further can reduce the power to detect true associations [10]. Several methods have been developed to correct the inflated false-positive rate caused by population stratification,

among which genomic control [11] and principal component analysis [12] are 2 popular approaches. The genomic control method assumes that population stratification will inflate the test statistics Y^2 from the χ^2_1 distribution expected under the null hypothesis (H_0) into a $\lambda\chi^2_1$ distribution, where λ is estimated as the mean value of Y^2 or the median value of Y^2 divided by 0.4549 (the expected median of a variable from the χ^2_1 distribution). The disadvantage of the genomic control method is that it assumes all genetic variants are influenced to the same degree by population stratification (i.e. λ is constant across different loci). In contrast, principal component analysis aims to infer differences in individuals' ancestry by summarizing genomic variation via the eigenvectors of the sample genotypic covariance matrix and can in turn allow the influence of population stratification to differ across variants.

Although it is clear that population stratification is a severe problem in association studies of common variants, little is known about the effect of population stratification on case-control studies of rare variants [13, 14]. Without correctly accounting for confounders such as population stratification, sequencing studies could potentially be plagued by false-positive results. Here, we use simulated sequence data based on coalescent models to examine whether population stratification affects studies of rare variants and whether existing methods can adequately adjust for this stratification.

Materials and Methods

Study Design and Notation

We assume a case-control study where N participants are sequenced for a region comprised of M variants. We define N_d and N_c as the number of cases and number of controls, respectively, with $N = N_d + N_c$. Let $\mathbf{D}$ be an $N \times 1$ column vector that denotes the disease status for the N research subjects, where $D_i = 1$ if subject i is a case, while $D_i = 0$ if the subject is a control ($i = 1, 2, \dots, N$). Define G as a $N \times M$ matrix comprised of the genotypes for the N subjects, where the i,j -th element of the matrix, g_{ij} , denotes the genotype of the i -th subject on the j -th locus ($j = 1, \dots, M$). We code g_{ij} to take the values 0, 1, or 2, representing the number of copies of the rare variant that the subject possesses at the locus. We define rare variants as those alleles with minor allele frequencies $\leq 1\%$ within the population.

Associations between Rare Variants and Disease

Collapsing Methods

Many statistical methods for rare-variant analysis collapse rare variants in a region together and analyze them as a group in order to improve the power to detect disease-associated alleles. In this paper, we consider 2 ways to form composite variables: the CMC method is based on the presence or absence of rare alleles in a region [8], while the burden test is based on the total number of rare

alleles in a region [9]. Assuming A regions are under study, we let C_{ia} denote the composite variable in region a ($a = 1, \dots, A$) for the i -th subject. For Li and Leal's CMC method [8]: $C_{ia} = 1$ if person i possesses rare variants in region a , and $C_{ia} = 0$ if there are no rare variants in the region. For the burden test [9]: C_{ia} = the total number of rare variants in region a ($a = 1, \dots, A$).

Test of Association

We used Pearson χ^2 tests and logistic regression to test whether the composite variables are significantly associated with the disease. When collapsing is based on the presence or absence of rare variants in the region, we can apply the Pearson χ^2 test statistic. Assuming equal numbers of cases and controls, the Pearson χ^2 statistics for the a -th region, X_a^2 , is defined as follows:

$$X_a^2 = N_d \left[\frac{[P_a^D(C_a = 0) - P_a^C(C_a = 0)]^2}{P_a^D(C_a = 0) + P_a^C(C_a = 0)} + \frac{[P_a^D(C_a = 1) - P_a^C(C_a = 1)]^2}{P_a^D(C_a = 1) + P_a^C(C_a = 1)} \right],$$

where $P_a^D(1, C_a = 1)$ and $P_a^C(C_a = 1)$ represent proportions of cases and controls with rare variants in the a -th region, while $P_a^D(C_a = 0)$ and $P_a^C(C_a = 0)$ represent proportions of cases and controls without rare variants in the a -th region, respectively.

When performing a burden test that counts the number of rare variants in a region, we use the score test based on logistic regression. The logistic regression model is defined as follows:

$$\text{logit}[E(D_i)] = \beta^T C_i,$$

where $C_i = (1, C_{i1}, C_{i2}, \dots, C_{iA})'$ are the composite variables and intercept term for the i -th subject. The logistic regression can accommodate non-binary independent variables but is equivalent to the Pearson χ^2 test shown above when the independent variable is binary.

Correction Method

We examined whether 2 methods that correct for population stratification in case-control studies of common variation (genomic control [11, 15] and principal component analysis [12, 16]) were as effective for rare-variant analyses using the CMC and burden methods described earlier. We describe each method in more detail below.

Genomic Control

As discussed by Devlin and Roeder [11] and Marchini et al. [15], genomic control is a popular method to adjust for population stratification in GWAS, though it has not been widely applied to studies of rare variants. This method is typically used when testing for association with a χ^2 test with 1 degree of freedom, such as the Pearson χ^2 test described above or the Cochran-Armitage trend test. The Cochran-Armitage test statistic for tests of common variants is defined as follows:

$$Y^2 = \frac{N \left[N(N_{dAa} + 2N_{dAA}) - N_d(N_{Aa} + 2N_{AA}) \right]^2}{N_d N_c \left[N(N_{Aa} + 4N_{AA}) - (N_{Aa} + 2N_{AA})^2 \right]},$$

where N_{dAa} and N_{dAA} are the number of SNPs in cases with 1 minor allele and 2 minor alleles, and N_{Aa} and N_{AA} are the total number of subjects with 1 minor allele and 2 minor alleles, respectively. Without stratification, under H_0 , the test statistic follows a χ^2 distribution with 1 degree of freedom. The genomic control method as-

sumes that, in the presence of stratification, test statistics will be inflated by a constant inflation factor λ , so that $Y^2 \sim \lambda \chi^2$. In a GWAS, the genomic control factor λ can be estimated as either the median of all observed χ^2 statistics divided by 0.4549 (the median of the χ^2_1 distribution) or as the mean value of all observed χ^2 statistics. In our simulated rare-variant analyses (described below), we estimate λ as the median test statistic from GWAS data on common variants divided by 0.4549. To apply genomic control to the CMC and burden tests, we calculate the inflation factor λ from the Cochran-Armitage trend test and then divide the observed CMC/burden test statistic by λ . For $\lambda \leq 1$, no adjustment is needed. We then calculate the p value of the adjusted CMC/burden test, assuming that the genomic control-adjusted test follows a χ^2 distribution with 1 degree of freedom.

Principal Component Analysis

Principal component analysis aims to summarize the variation in a dataset as a sequence of uncorrelated components, which are linear combinations of the variables in the original dataset. The p -th component can be summarized as follows:

$$P_p = B_p' G = b_{p1} g_1 + b_{p2} g_2 + \dots + b_{pM} g_M,$$

where $B_p = (b_{p1}, b_{p2}, \dots, b_{pM})'$ is a $M \times 1$ vector and g_j represents the j -th column of G . The principal components are ordered by their ability to summarize the data. As a result, the first component, P_1 , accounts for as much of the variation in G as possible for a linear combination of the variables; the second component, P_2 , accounts for as much as possible of the remaining variation of G , and so on. To calculate the principal-component coordinates for each subject, we first subtracted the empirical column mean μ_j calculated as:

$$\mu_j = \frac{1}{N} \sum_{i=1}^N G_{ij},$$

from each column j of G . We then divided each entry by its empirical column standard deviation, SD_j . We use S to denote the standardized G matrix with $S_{ij} = (G_{ij} - \mu_j)/SD_j$. As discussed in Price et al. [12], B_p is the coordinate of the p -th eigenvector of the variance-covariance matrix of S . We define V as the $M \times M$ variance matrix of S , where element $V_{jj'}$ represents the covariance between locus j and locus j' . We used the singular value decomposition method to compute the eigenvectors of V . The singular value decomposition method can decompose S into the product of 3 matrices: $S = U \Sigma W^T$, where U is a matrix whose columns are eigenvectors of the matrix SS^T , Σ is a $N \times M$ diagonal matrix with the values on the diagonal being the singular values of S , and columns in W are eigenvectors of the matrix $S^T S$. As SS^T is equivalent to the variance-covariance matrix V , the p -th column of U contains the coordinates of the p -th principal component for the subjects in the sample. To use the principal component method to correct for population stratification in our simulations described below, we include the top principal components as additional covariates in the logistic regression model:

$$\text{logit}[E(D_i)] = \beta^T C_i + \gamma^T P_i,$$

where D_i denotes the disease status for the i -th subject, $C_i = (1, C_{i1}, C_{i2}, \dots, C_{iA})'$ are the composite variables for the i -th subject and P_i denotes the vector of the top principal components.

Simulations

Type I Error

To test the performance of the methods described above, we simulated case-control resequencing datasets subjected to confounding due to population stratification. To generate realistic resequencing data, we used *cosi* [17] to generate 250-kb haplotypes for 20,000 European and 20,000 African individuals. As discussed in Schaffner et al. [17], *cosi* can simulate haplotypes with high resemblance to empirical data collected by the International HapMap Project [18]. We first used *cosi* to generate haplotypes under the ‘best fit model’ as described in Schaffner et al. [17]. We also simulated haplotypes under an exponential growth model, where we assume that from the 50th to the 1,000th generation the European population increased in size from 8,000 to 100,000 people, while the African population increased from 10,000 to 100,000 people, based on common assumptions about effective population sizes [19]. We used R to simulate case-control studies with population structure as follows: we set the number of European versus African individuals to be 1:1 in controls and held this constant for all studies. However, we allowed the proportion of European and African individuals to vary in cases. We simulated across cases in 4 different proportions: (1) 50% Europeans versus 50% Africans; (2) 40% Europeans versus 60% Africans; (3) 25% Europeans versus 75% Africans, and (4) 10% Europeans versus 90% Africans. Three different sample sizes were used: 100 cases:100 controls, 500 cases:500 controls, and 1,000 cases:1,000 controls. For each scenario considered, we performed 500 simulations. For all analyses, we assumed a significance threshold of $\alpha = 0.05$.

Power

To evaluate power, we generated case-control datasets prospectively, assuming the odds of a disease is a function of ancestry (European/African) and rare causal variants:

$$E(D) = \frac{\exp(\beta_0 + \theta \times I(\text{African}) + \eta)}{1 + \exp(\beta_0 + \theta \times I(\text{African}) + \eta)},$$

where β_0 is the prevalence of the disease, θ is the odds ratio of the disease risk between African and European subjects, I is an indicator function, and η is the odds ratio of causal rare variants. For each model considered, we performed 300 simulations. For all analyses, we assumed a significance threshold of $\alpha = 0.05$.

Generating Additional Markers for Stratification Adjustment

We next generated SNP data to use for stratification adjustment of the CMC and burden tests in the presence of population stratification. Since genomic control and principal component analysis require far more SNPs for an accurate correction than those found in a simulated region, we instead generated genome-wide SNP data using HapMap markers found on the Affymetrix 6.0 array SNPs. We used such HapMap SNP data from the Yoruban (YRI) and CEPH (CEU) populations to represent African and European populations, respectively. We performed LD-based pruning in PLINK [20] to filter out SNPs in strong LD ($R^2 \geq 0.5$). We used the minor allele frequencies of the remaining SNPs to generate genotype data under the assumption of Hardy-Weinberg equilibrium. We used the minor allele frequencies from the HapMap CEU samples to generate genotypes for European subjects and the minor allele frequencies from the HapMap YRI samples to generate genotypes for African subjects. We assumed that these

SNPs follow Hardy-Weinberg equilibrium and simulated genotypes based on the binomial distribution.

We next used the SNP genotype data to correct for population structure in our case-control study. In each simulation, we randomly selected a simulated GWAS genotype for each subject in our simulated case-control study, selecting from the set of African or European genotypes depending on the ancestry of each subject. Using the simulated data, we analyzed the GWAS data to calculate the inflation factor and to construct principal components using the methods discussed above. As there are only 2 populations (African and European) in our simulation, we only included the first and the second principal components in our logistic regression models; as a result, P is an $N \times 2$ matrix. We then evaluated the type I error rates and power of the CMC method [8] and burden test [9] when adjusting for population stratification with genomic control or principal components.

Results

We first performed simulation studies under H_0 of no association between rare variants and the disease to assess whether population stratification can lead to spurious associations in resequencing studies. We simulated case-control studies subject to different stratification levels for various sample sizes (as described in Materials and Methods). We first used the best fit model of *cosi* [17] to simulate 20,000 European and 20,000 African haplotypes that have high resemblance to HapMap data. For each simulation, we randomly selected a 10-kb region as the targeted region to study the association between genetic variants in the region and disease status. As described in Materials and Methods, we fitted logistic regression models using both the CMC [8] and burden methods [9] in each of 500 simulations and estimated the type I error rate as the proportion of simulations with a significant association ($p < 0.05$). The type I error rates with different sample sizes and at different stratification levels are shown in figure 1. The type I error rate increases as the stratification level increases. When there is no stratification (50% Africans vs. 50% Europeans in both cases and controls), the type I error rate is around 0.05; however, when population stratification exists, the type I error rate is inflated up to 0.56 for the CMC method and 0.59 for the burden test. The inflation of the type I error rate becomes larger as the sample size increases. For large samples (1,000 cases:1,000 controls), even a modest stratification (60% Africans vs. 40% Europeans in cases) leads to an inflated type I error rate around 0.2. This is because the type I error rate under H_0 is actually the power to detect any association with the disease, even a spurious association due to population stratification. As a result, the type I error rate goes up as

Fig. 1. Type I error rates for the CMC and burden tests uncorrected or corrected by principal component analysis and genomic control for a 10-kb region. 500 cases and 500 controls collapsed by the CMC method (**a**) and by the burden test (**b**). 1,000 cases and 1,000 controls collapsed by the CMC method (**c**) and by the burden test (**d**). Note that in all simulations, 50% of controls have African ancestry and 50% have European ancestry, while the proportion of cases with African ancestry varies across the simulations (x-axis).

the sample size goes up. The CMC method and the burden test have a similar performance, although the burden test has systematically higher type I error rates compared to the CMC method.

To examine the effect of population stratification on rare variation under different population-genetics models, we next simulated the European and the African haplotypes under the exponential growth model as described earlier. Overall, we observed no marked difference in the results generated under the exponential growth model compared to the results generated under the best fit model [17]. For the exponential growth model, when there are 900 Africans and 100 Europeans in cases (500 Africans and 500 Europeans in controls), the type I error rate is 0.55 for the CMC method and 0.57 for the burden test. These results are quite similar to the best fit results under the same stratification model. Thus, our results appear to be consistent under different population-genetics models.

To examine how the type I error rate is influenced by the number of variants in a region, we also performed simulations for 1- and 50-kb regions. As the region size increased, we found that the behavior of the rare-variant tests depended on the collapsing method used (table 1). Interestingly, when stratification existed but was not adjusted for, we observed that the CMC method demonstrated less type I error rate inflation for a 1-kb region or a 50-kb region compared to a 10-kb region. On the other hand, the burden test demonstrated greater type I error rate inflation with increasing region size. While counter-intuitive, we believe that the type I error rate for the CMC method is the most inflated for a 10-kb region because the CMC method is based on the presence/absence of rare variants in a region. As the region size increases, more individuals will have at least 1 rare variant in a region, even when stratification exists. Online supplementary table 1 (see www.karger.com/doi/10.1159/000353270)

Table 1. Type I error rates for the CMC and burden tests before and after correction by principal component analysis (PCA) and genomic control (GC) based on 1,000 cases and 1,000 controls

Cases that are of African ancestry, %	Region size kb	Before correction		After correction (PCA)		After correction (GC)	
		CMC	burden	CMC	burden	CMC	burden
50	1	0.064	0.068	0.064	0.058	0.064	0.068
	10	0.045	0.056	0.055	0.060	0.045	0.056
	50	0.064	0.075	0.058	0.062	0.064	0.075
90	1	0.444	0.426	0.046	0.046	0	0
	10	0.580	0.610	0.070	0.060	0	0
	50	0.240	0.670	0.060	0.097	0	0

Simulations assume 50% of controls have African ancestry.

Table 2. Type I error rates for common variants compared to rare variants collapsed by the CMC or burden tests

Cases that are of African ancestry, %	Correction	Rare variants (CMC)	Rare variants (burden)	Common variants
50	before	0.045	0.056	0.036
	PCA	0.055	0.060	0.032
	GC	0.045	0.056	0.036
90	before	0.580	0.610	0.870
	PCA	0.070	0.060	0.036
	GC	0	0	0.010

Rates are presented before and after correction by principal component analysis (PCA) and genomic control (GC). Simulations are based on 10-kb regions using 1,000 cases and 1,000 controls. All simulations assume 50% of controls have African ancestry.

demonstrates that, in our simulations, the majority of individuals have 0 rare variants in a given 1-kb region, while the majority of individuals have >0 rare variants in a given 50-kb region. In contrast, the average number of individuals with 0 versus those with >0 rare variants is roughly evenly distributed for regions of 10 kb. Consequently, our ‘power’ to detect population stratification is greatest for the 10-kb regions, while the effect of stratification is attenuated for the 1- and 50-kb regions. The burden test, on the other hand, is based on the total number of rare variants in a region and can take a larger range of values. Thus, we expect the type I error rate inflation of this test to be exacerbated with increasing region size.

We next examined whether genomic control and principal components can correct for the confounding due to population stratification in the samples. For the genomic control method (fig. 1, diamonds), the results are very conservative. When the stratification level is a bit more severe (75% Africans vs. 25% Europeans), using genomic control will lead to a type I error rate close to 0. This can seriously reduce power in the presence of true association. However, the principal component method performed very well (fig. 1, squares): the type I error rates generally are distributed around 0.05, although there is a slight type I error rate elevation under the extreme stratification model. We examined the same pattern for the 1-kb and the 50-kb region (table 1). In general, principal component correction works better for the CMC method compared to the burden test, especially when the region size is large (fig. 1; table 1).

As a comparison, we also applied these 2 correction methods to a common-variant simulation of similar frequency. For each simulation, we calculated the average frequency of the composite variable after collapsing the rare variants in the region using the CMC method. In each simulation, we then selected the common variant with an allele frequency most similar to the composite variable and computed the type I error based on these common variants. In the presence of population stratification, the common variant has a much higher type I error rate than either the CMC or burden tests of rare variants (table 2). Genomic control is also very conservative in the common-variant simulations, although not as conservative as for rare variants. Principal component analysis, on the other hand, has a much better performance.

Fig. 2. The power of rare-variant sequencing studies subject to population stratification. θ (the odds ratio of the disease risk between African and European subjects) is 1 (**a**) and 4 (**b**), collapsed by the CMC method. θ is 1 (**c**) and 4 (**d**), collapsed by the burden test.

We next performed power calculations to examine the performance of these 2 correction methods in the presence of true association between rare variants and the disease. Here we used the haplotypes generated by *cosi* to simulate case-control studies subjected to population stratification. For each simulation, we randomly selected a specified number of African and European haplotypes and randomly selected a 10-kb region as the targeted region. We then generated the case/control status prospectively, such that the odds of having the disease are a function of both rare causal variants and ancestry (European/African). For our simulations, we assumed that the baseline prevalence of the disease is 0.05 and the probability that a rare variant is causal is 0.3. We allowed Africans to have a 4-fold increased odds of being a case compared to Europeans; as a comparison, we also considered a separate simulation assuming equal disease odds for Africans and Europeans. We simulated disease outcomes such that

the presence of causal rare variants leads to a 1- to 5-fold increase in the risk of disease. The simulation results based on 300 simulations are summarized in figure 2. We do not include the uncorrected results in figures 2b and d because table 1 and figure 1 have shown that it is necessary to adjust for population stratification to avoid spurious results due to an inflated false-positive rate. This shows that it is necessary to adjust for population stratification to avoid spurious results due to an inflated false-positive rate. Consistent with figure 1, genomic control regularly leads to extremely conservative results, while principal component analysis controls type I error at the target level. Although power increases with the odds ratio of causal rare variants, the power remains close to 0 when using genomic control as a correction method and collapsing by CMC [8]. As in figure 1, the burden test and CMC method have a very similar performance.

Discussion

In this paper, we showed that population stratification can lead to an inflated type I error rate in rare-variant association studies. We examined 2 methods to correct for this stratification: principal component analysis and genomic control. Our simulations showed that principal component analysis could control the false-positive rate at the desired level, while maintaining power to identify true associations. Genomic control, on the other hand, leads to extremely conservative results in this setting. Using genomic control as a correction method in resequencing studies will lead to substantially lower power to detect association. Our analysis also examined 2 methods of collapsing rare variants: the CMC method by Li and Leal [8] and the burden test by Morris and Zeggini [9]. Our simulation results suggested that the degree of inflation due to population stratification depended on the number of variants considered (i.e. the region size) and the specific collapsing method used; correction with principal component analysis was more successful for the CMC method than for the burden test. A recent paper by Mathieson and McVean [21] also investigated the impact of confounding due to population stratification on rare-variant and com-

mon-variant tests, but this work focused on spatially structured, rather than discrete, populations.

Our results imply that researchers need to carefully match cases and controls on ancestry to avoid population stratification in studies of rare variants. Although principal component analysis can provide the desired correction, it effectively relies on genome-wide data, which may not always be available, such as in targeted sequencing studies like the Dallas Heart Study. Consequently, the only way to ensure robustness of rare-variant association tests may be to use family-based studies and implement methods similar to the transmission disequilibrium test for analysis. We will explore such designs in a future paper.

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