

Optimal unified approach for rare variant association testing with application to small sample case-control whole-exome sequencing studies

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Abstract

We propose in this paper a unified approach for testing the association between rare variants and phenotypes in sequencing association studies. This approach maximizes power by adaptively using the data to optimally combine the burden test and non-burden sequence kernel association test (SKAT). Burden tests are more powerful when most variants in a region are causal and the effects are in the same direction, while SKAT is more powerful when a large fraction of the variants in a region are non-causal or the effects of causal variants are in different directions. The proposed unified test is hence optimal in both scenarios. We show that the unified test corresponds to the optimal test in an extended family of SKAT tests, which we refer to as SKAT-O. The second goal of this paper is to develop a small sample adjustment procedure for the proposed methods to correct conservative type I error rates of SKAT-family tests when the trait of interest is dichotomous and the sample size is small. Both small sample adjusted SKAT and the unified test (SKAT-O) are computationally efficient and can be easily applied to genome-wide sequencing association studies. We evaluate the finite sample performance of the proposed methods using extensive simulation studies and illustrate their application using the acute lung injury exome sequencing data of the NHLBI exome sequencing project.

1 Introduction

Array-based genotyping technologies have been successfully used in hundreds of genome-wide association studies in the last few years for identifying over a thousand common genetic variants associated with many complex diseases. The recent advance of massively parallel sequencing technologies^{1,2} has transformed human genetic research. These emerging sequencing technologies provide a rich opportunity to study the association between rare variants and complex traits. Rare variants, which have minor allele frequencies (MAFs) less than $0.01 \sim 0.05$, might play an important role in the etiology of complex traits and account for missing heritability unexplained by common variants.^{3,4} Several complex traits have been found to be associated with rare variants.^{5,6,7}

In recent years, significant efforts have been devoted to developing powerful and computationally efficient statistical methods to test associations between rare variants and complex traits. While single variant tests are typically conducted to investigate associations of common variants and phenotypes, the same approach has little power for testing for rare variant effects due to their low frequencies and large numbers. Instead, the statistical development of rare variants analysis has been focused on testing cumulative effects of rare variants in genetic regions or SNP sets, such as genes. These tests can be broadly classified as burden and non-burden tests.

Burden tests collapse rare variants in a genetic region into a single burden variable, and then regress the phenotype on the burden variable to test for the cumulative effects of rare variants in the region. Examples of the burden tests include the cohort allelic sum test (CAST),⁸ the combined multivariate and collapsing method (CMC),⁹ and non-parametric weighted sum test (WST),¹⁰ which imposes weights when collapsing rare variants. Several alternative burden methods are largely based on the same approach.^{11,12,13,14} Since all burden tests implicitly assume that all the rare variants in a region are causal and affect the phenotype in the same direction with similar magnitudes, they suffer from a substantial loss of power when these assumptions are violated.^{15,16}

Kernel based test methods, such as the sequence kernel association test (SKAT),¹⁷ are non-burden tests. Instead of aggregating variants, SKAT aggregates individual variant score test statistics with weights when SNP effects are modeled linearly. More generally, SKAT aggregates the associations between variants and the phenotype through a kernel matrix and can allow for SNP-SNP interac-

tions, i.e., epistatic effects. SKAT is especially powerful when a genetic region has both protective and deleterious variants or many non-causal variants. SKAT is derived as a variance component test in the induced mixed models when regression coefficients are assumed to be independent and follow a distribution with variance component. SKAT efficiently calculates the p-value analytically for large samples, and is hence computationally fast for analyzing genome-wide sequencing association studies. It has been shown that some non-burden tests such as the C-alpha test¹⁵ are a special case of SKAT.^{17,16}

Although SKAT provides attractive power and makes few assumptions about the rare variant effects, it has several limitations. It can be less powerful than burden tests if a large proportion of the rare variants in a region are truly causal and influence the phenotype in the same direction.^{16,17} In addition, large sample based p-value calculations using SKAT can produce conservative type I errors for small sample case/control sequencing association studies, which could lead to power loss.^{17,18} This is particularly an issue in current exome sequencing studies, which are often of small sizes.

This paper aims to address the limitations of burden tests and SKAT, and has two objectives. First, we propose a unified test for rare variant effects by using the data to find the optimal linear combination of the burden test and SKAT to maximize the power. We show that this unified test belongs to an extended SKAT test family by allowing the regression coefficients of variants to be correlated.¹⁹ We hence term this optimal unified test as SKAT-O, which is optimal in both scenarios. Specifically, using the data, it automatically behaves like the burden test in the situation when the burden test is more powerful than SKAT, and behaves like SKAT in the situation when the SKAT is more powerful than the burden test.

The second objective of this paper is to improve the performance of SKAT and SKAT-O in small sample case-control sequencing association studies. The original SKAT test has been found to be conservative for small samples.^{17,18} In this paper we develop an analytic adjustment method for SKAT and SKAT-O by estimating the small sample variance and kurtosis precisely. This allows us to precisely calculate the reference distribution for a small sample, thereby properly controlling the type I error. This is motivated by the fact that many of the current exome sequencing studies, such as those in the NHLBI Exome Sequencing Project, have small sample sizes, e.g., the Acute Lung Injury (ALI) exome sequencing data that are discussed in this paper have 88 subjects. The proposed small sample

adjustment method is computationally fast and can be effectively applied to whole exome(genome) sequencing studies.

Using extensive simulations and analysis of the ALI exome sequencing data of the NHLBI Lung GO Exome Sequencing Project (ESP), we demonstrate that the small sample adjusted unified test (SKAT-O) has proper type I error rates for small sample sequencing association studies, has higher power in a wide range of settings and is more robust than SKAT and the burden tests.

2 Methods

For simplicity, we assume that we are interested in testing the association between rare variants in a region, e.g., a gene, and a complex trait. For whole exome sequencing studies (WES) and whole genome sequencing studies (WGS), the same method can be applied to one gene or one region at a time and then adjusted for multiple comparisons by the user's method of choice. For WGS, one can consider analysis of one window of the same size, e.g., 30kb, at a time using the moving window approach.

2.1 Sequence Kernel Association Test

Assume n subjects are sequenced in a region, e.g., a gene, that has m variants. For the i^{th} subject, let y_i denote a dichotomous phenotype, $\mathbf{G}_i = (g_{i1}, \dots, g_{im})'$ the genotypes of the m variants ($g_{ij} = 0, 1, 2$), $\mathbf{X}_i = (x_{i1}, \dots, x_{is})'$ the covariates. Without loss of generality, we assume an additive genetic model and a binary trait. Results are similar for quantitative traits. To relate genotypes to a dichotomous phenotypes, we consider the logistic regression model

$$\text{logit}(\pi_i) = \gamma_0 + \mathbf{X}_i' \boldsymbol{\gamma}_1 + \mathbf{G}_i' \boldsymbol{\beta}, \quad (1)$$

where π_i is the disease probability, $\boldsymbol{\gamma}_1$ is an $s \times 1$ vector of regression coefficients of covariates, and $\boldsymbol{\beta} = (\beta_1, \dots, \beta_m)'$ is an $m \times 1$ vector of regression coefficients of genetic variants. The standard m degrees of freedom (DF) test for no genetic association $H_0: \boldsymbol{\beta} = 0$ has little statistical power when m is large. Several approaches have been proposed to reduce the DF and increase analysis power. Two

classes of tests have been proposed: burden and non-burden tests.

Burden tests treat the β_j 's to be the same up to a weight function, i.e., $\beta_j = w_j\beta_c$, where w_j is a weight function that may depend on properties of the j^{th} variant. For example, one can assume w_j to be a function of Minor Allele Frequency (MAF). Then (1) becomes

$$\text{logit}(\pi_i) = \gamma_0 + \mathbf{X}_i' \gamma_1 + \beta_c \left\{ \sum_{j=1}^m w_j g_{ij} \right\}, \quad (2)$$

and the association between the m genetic variants and a dichotomous trait can be tested using a one DF test for $H_0: \beta_c = 0$. Suppose $\hat{\pi}_i$ is the estimated probability of y_i under the null hypothesis, i.e., $\hat{\pi}_i$ is calculated by fitting the null model

$$\text{logit}(\pi_i) = \gamma_0 + \mathbf{X}_i' \gamma_1. \quad (3)$$

Then the burden score statistic for testing $H_0: \beta_c = 0$ is

$$Q_B = \left[\sum_{i=1}^n (y_i - \hat{\pi}_i) \left(\sum_{j=1}^m w_j g_{ij} \right) \right]^2, \quad (4)$$

which asymptotically follows scaled χ_1^2 under the null hypothesis. This weighted burden test is equivalent to the weighted sum test of Madsen and Browning¹⁰ and Han and Pan,¹³ where Madsen and Browning¹⁰ assumed $w_j = 1/\sqrt{\tilde{p}_j(1-\tilde{p}_j)}$, where \tilde{p}_j is the estimated MAF for SNP j using controls. When all w_j are the same and analysis is restricted to rare variants, e.g., the variants with $\text{MAF} < 5\%$, Q_B is equivalent to the Morris and Zeggini (MZ) test.¹² The key limitation of the weighted burden test is that it assumes all rare variants in the region are causal and are associated with the trait in the same direction with the same magnitude after weighting, and thus the presence of both protective and deleterious variants or a large number of non-causal variants would substantially reduce its statistical power.

SKAT,¹⁷ which includes the C-alpha test¹⁵ as a special case, is a non-burden test. SKAT assumes that the β_j in (1) are independent and follow an arbitrary distribution with mean 0 and variance $w_j^2 \tau$.

The null hypothesis $H_0 : \beta = 0$ in model (1) is equivalent to the hypothesis $H_0 : \tau = 0$. Hence SKAT is a variance component test under the induced logistic mixed model.²⁰ Specifically, under the logistic model (1), the SKAT statistic can be written as

$$Q_S = (\mathbf{y} - \hat{\boldsymbol{\pi}})' \mathbf{K} (\mathbf{y} - \hat{\boldsymbol{\pi}}), \quad (5)$$

where $\hat{\boldsymbol{\pi}} = (\hat{\pi}_1, \dots, \hat{\pi}_n)'$ is a vector of the estimated probability of \mathbf{y} under the null model (3), and $\mathbf{K} = \mathbf{G} \mathbf{W} \mathbf{W} \mathbf{G}'$ is an $n \times n$ kernel matrix, where $\mathbf{G} = (\mathbf{G}_1, \dots, \mathbf{G}_n)'$ is an $n \times m$ genotype matrix, and $\mathbf{W} = \text{diag}(w_1, \dots, w_m)$ is an $m \times m$ diagonal weight matrix. The SKAT statistic Q_S can be simplified as the weighted sum of the individual SNP score statistics as

$$Q_S = \sum_{j=1}^m w_j^2 S_j^2 = \sum_{j=1}^m w_j^2 \left\{ \sum_{i=1}^n g_{ij} (y_i - \hat{\pi}_i) \right\}^2, \quad (6)$$

where $S_j = \sum_{i=1}^n g_{ij} (y_i - \hat{\pi}_i)$ is the score statistic for testing $H_0 : \beta_j = 0$ in the individual SNP j only model

$$\text{logit}(\pi_i) = \gamma_0 + \mathbf{X}_i' \boldsymbol{\gamma} + g_{ij} \beta_j.$$

The weight w_j can be flexibly chosen using the observed data, such as a function of MAF, or external information, such as PolyPhen or SIFT score.^{21,22} For example, Wu *et al.* (2011)¹⁷ proposed to use a Beta density function of MAF as a weight function in which $w_j = \text{Beta}(p_j, a_1, a_2)$, where p_j is the estimated MAF for SNP j using all cases and controls, and the parameters a_1 and a_2 are pre-specified and q_j is estimated from all samples. The SKAT test statistic Q_S asymptotically follows a mixture of chi-square distributions.¹⁷ For large samples, the p-value of SKAT can be quickly and accurately calculated by either matching the moments or inverting the characteristic function.^{23,24,25}

A comparison of the burden statistic Q_B in (4) and the SKAT statistic Q_S in (6) shows that the burden test aggregates the variants first before performing regression, while SKAT aggregates individual variant test statistics. Hence SKAT is robust to the mixed signs of β 's and a large fraction of non-causal variants.

2.2 Optimal Unified association test

The foregoing discussions suggest that burden tests are not powerful when the target region has many non-causal variants or causal variants have different directions of association, while SKAT is powerful in these situations.¹⁷ However, if the target region has a high proportion of causal variants with the effects in the same direction, burden tests can be more powerful than SKAT. Because such prior biological knowledge is often unknown, and the underlying genetic mechanisms vary from one gene to another across the genome, it is of substantial interest to develop a test that is optimal for both scenarios in whole exome (genome) sequencing studies. Here we propose a unified test that includes burden tests and SKAT in one framework. In particular, the test statistic of the proposed unified test is

$$Q_\rho = \rho Q_B + (1 - \rho) Q_S, \quad 0 \leq \rho \leq 1, \quad (7)$$

which is a weighted average of SKAT and burden test statistics. One can easily see that the unified test reduces to SKAT when $\rho = 0$ and to the burden test when $\rho = 1$, i.e., the class of tests Q_ρ ($0 \leq \rho \leq 1$) includes the burden test and SKAT as special cases. One can further show that the unified test (7) is equivalent to the generalized SKAT test¹⁹ derived as the variance component score statistic assuming the regression coefficients β_j in (1) follow an arbitrary distribution with mean 0 and variance $w_j^2 \tau$ and pairwise correlation ρ between different β_j 's as

$$Q_\rho = (\mathbf{y} - \hat{\boldsymbol{\pi}})' \mathbf{K}_\rho (\mathbf{y} - \hat{\boldsymbol{\pi}}), \quad (8)$$

where $\mathbf{K}_\rho = \mathbf{G} \mathbf{W} \mathbf{R}_\rho \mathbf{W} \mathbf{G}'$ is an $n \times n$ kernel matrix, $\mathbf{R}_\rho = (1 - \rho) \mathbf{I} + \rho \mathbf{1} \mathbf{1}'$ is an $m \times m$ compound symmetric matrix, and $\mathbf{1} = (1, \dots, 1)'$. This implies that the weight ρ in (7) can be interpreted as the correlation of the regression coefficients β_j 's ($j = 1, \dots, m$). If the regression coefficients β_j are perfectly correlated ($\rho = 1$), they will be all the same after weighting, and one should collapse the variants first before running regression, i.e., using the burden test. If the regression coefficients are unrelated to each other, one should use SKAT.

In practice, the optimal weight ρ is unknown and needs to be estimated from the data to maximize the power. Lee *et al.* (2011)¹⁹ proposed the optimal test procedure for the generalized SKAT, which

selects the weight ρ to maximize the power. It follows that the optimal unified test can be calculated as

$$Q_{optimal} = \min_{0 \leq \rho \leq 1} p_{\rho}, \quad (9)$$

where p_{ρ} is the p-value computed based on a given ρ . The optimal unified test statistic can be easily obtained by the simple grid search: set a grid $0 = \rho_1 < \rho_2 < \dots < \rho_b = 1$, then

$$Q_{optimal} = \min\{p_{\rho_1}, \dots, p_{\rho_b}\}.$$

For large samples, Lee *et al.*(2011)¹⁹ showed that for given ρ , each test statistic Q_{ρ} can be decomposed into a mixture of two random variables, one asymptotically follows a chi-square distribution with one DF, and the other can be asymptotically approximated to a mixture of chi-square distributions with a proper adjustment. Hence the p-value of $Q_{optimal}$ can be quickly obtained analytically using a one-dimensional numerical integration. We term the optimal unified test as SKAT-O in view of the fact that it is an optimal test in the generalized SKAT family.

2.3 Small sample optimal unified test

One of the key strengths of SKAT and SKAT-O over the other competing methods is their ability to efficiently compute asymptotic p-values without the need for resampling, and it is easy to adjust for covariates. This is particularly advantageous in whole genome (exome) sequencing studies when a large number of tests is performed and one needs to control for multiple comparisons and account for population stratification. However, if the trait is binary and sample sizes are small, e.g., hundreds of subjects, the large sample based p-value calculations in Wu *et al.*(2011)¹⁷ and Lee *et al.*(2011)¹⁹ have been found to produce conservative results, which can lead to incorrect type I error control and power loss.^{17,19,18}

As most current whole exome sequencing studies, such as the those of NHLBI Exome Sequencing Project, have small sample sizes, there is a pressing need to develop a method that works well for small samples. We propose in this section small sample adjusted p-value calculations for SKAT and SKAT-O.

We first consider p-value calculations for SKAT when sample sizes are small. When variants are

rare, the genotype matrix \mathbf{G} is sparse, and the small sample variance of Q_S is much smaller than the asymptotic variance. Hence, we re-adjust the moments of the null distribution of Q_S . Suppose Q_S was obtained with known π . Denote by $\mathbf{D} = \text{diag}\{\pi_i(1 - \pi_i)\}$, where π_i is the probability of being a case under the null. Let $\tilde{\mathbf{K}} = \mathbf{D}^{1/2}\mathbf{K}\mathbf{D}^{1/2}$, $\mathbf{\Lambda} = \text{diag}\{\lambda_1, \dots, \lambda_q\}$ be a diagonal matrix of ordered non-zero eigenvalues, $\mathbf{U} = [\mathbf{u}_1, \dots, \mathbf{u}_q]$ be an $n \times q$ eigenvector matrix of $\tilde{\mathbf{K}}$, and u_{ij} be the i^{th} element of \mathbf{u}_j . In the Appendix, we show that the small sample mean and variance of SKAT under the null hypothesis are

$$E[Q_S|\mathbf{U}, \mathbf{\Lambda}, \pi] = \sum_{j=1}^q \lambda_j \quad \text{and} \quad \text{Var}[Q_S|\mathbf{U}, \mathbf{\Lambda}, \pi] = \sum_{j=1, k=1}^q \lambda_j \lambda_k c_{jk}, \quad (10)$$

where

$$c_{jk} = \sum_{i=1}^n \frac{u_{ij}^2 u_{ik}^2 (3\pi_i^2 - 3\pi_i + 1)}{\pi_i(1 - \pi_i)} + \sum_{i_1 \neq i_2}^n u_{i_1 j}^2 u_{i_2 k}^2 + 2 \sum_{i_1 \neq i_2}^n u_{i_1 j} u_{i_2 j} u_{i_1 k} u_{i_2 k} - 1.$$

A comparison of these results with those in Wu *et al.* (2011)¹⁷ shows that the small sample mean of Q_s is the same as the asymptotic mean of Q_S but the small sample variance differs from the asymptotic variance. Using the estimated moments, the p-value can then be calculated as

$$1 - F((Q_S - \mu_Q) \sqrt{2df} / \sqrt{v_Q} + df | \chi_{df}^2), \quad (11)$$

where $F(\cdot | \chi_{df}^2)$ is the distribution function of χ_{df}^2 , and

$$\mu_Q = \sum_{j=1}^q \lambda_j, \quad v_Q = \sum_{i,j=1}^q \lambda_i \lambda_j \hat{c}_{jk}, \quad \text{and} \quad df = \frac{\sum_{j=1}^q \lambda_j^{*4}}{(\sum_{j=1}^q \lambda_j^{*2})^2},$$

and $\lambda_j^* = \lambda_j \hat{c}_{jk} / \sqrt{2}$. \hat{c}_{jk} is an estimated c_{jk} with $\hat{\pi}$. We can apply the same approach to the optimal unified test SKAT-O; details are shown in the Appendix.

2.4 Small sample SKAT and unified test with higher moments adjustments

In the previous section, we adjusted the asymptotic null distribution of Q_S and Q_{optimal} using the small sample variance of Q_S and Q_{optimal} . If the sample size is very small, e.g., $n = 88$ in the ALI whole exome sequencing data, this approach may not be accurate enough to correct small sample type

I error rates. We thus need to adjust higher moments, especially kurtosis. Unfortunately, deriving the analytical formula of the kurtosis of Q_S is a daunting task. Hence we propose a practical approach in which the kurtosis is estimated through a re-sampling method. When there is no covariate, the kurtosis of the null distribution of Q_S can be estimated from B permutation samples of phenotypes, and then the estimated kurtosis can be used to calculate the degrees of freedom parameter (df) in (11).

Specifically, suppose $Q_{s,b}^*$ ($b = 1, \dots, B$) is the SKAT test statistic from the permutation sample \mathbf{y}_b^* . The sample kurtosis is

$$\hat{\gamma} = \frac{\hat{\mu}_4}{\hat{\sigma}^4} - 3,$$

where

$$\hat{\mu}_4 = \frac{1}{B} \sum_{b=1}^B (Q_{s,b}^* - \mu_Q)^4, \quad \text{and} \quad \hat{\sigma}^2 = \frac{1}{B} \sum_{b=1}^B (Q_{s,b}^* - \mu_Q)^2.$$

The degrees of freedom of the mixture of chi-square distribution (df) in (11) is modified as

$$df = 12/\hat{\gamma},$$

and the p-values can be calculated using the (11).

When there are covariates to adjust for, the simple permutation method cannot be used. Instead we propose to generate re-sampled phenotypes from the parametric bootstrap.²⁶ We first estimate π_i under the null model and use it to generate \mathbf{y}_b^* with the same number of cases and controls.

It should be noted that our method has a computation time advantage over calculating p-values based on permutations or bootstrap samples that are obtained as a proportion of $Q_{s,b}^*$ larger than Q_S . For whole exome sequencing studies, one needs to calculate p-values at the $10^{-5} \sim 10^{-6}$ level to account for multiple comparison adjustments for performing tests for 20,000 genes. This requires more than $10^7 \sim 10^8$ permutations or bootstraps for each gene. However, our approach requires sampling phenotypes only 10,000 times to obtain stable estimates of the higher moments. We hence can save a substantial amount of computation time.

2.5 Numerical Simulations

We conducted extensive simulation studies to evaluate the performance of the proposed methods for binary traits when sample sizes are small. We generated sequence data of European ancestry from 10,000 chromosomes over 1 Mb regions using the calibrated coalescent model.²⁷ Since the average length of exomes in a gene is around 3Kb, we randomly selected regions with length 3Kb, and tested for associations in all simulation settings.

2.5.1 Type I Error Simulations

We first generated datasets under the null model to evaluate the type I error control of the proposed methods. Dichotomous phenotypes with 50% cases and 50% controls were generated from the null logistic regression model

$$\text{logit}(\pi_i) = \gamma_0 + 0.5X_{1i} + 0.5X_{2i},$$

where X_1 was a continuous covariate from $N(0, 1)$, X_2 was a binary covariate from $Bernoulli(0.5)$, and γ_0 was chosen to create a trait prevalence of 0.01 under the null hypothesis. We applied six different methods to each of the randomly selected 3 Kb regions: 1) counting based burden test (N); 2) weighted burden test (W); 3) SKAT without small sample adjustment (SKAT); 4) unified test without small sample adjustment (SKAT-O); 5) small sample adjusted SKAT (adjusted SKAT); and 6) small sample adjusted unified test (adjusted SKAT-O).

For all methods except N, $Beta(1, 25)$ weights were used to upweight rarer variants. For N, we used flat weights and restricted variants with observed MAF < 0.03 . For both N and W, the likelihood ratio test was used to compute p-values. The p-values of the optimal unified tests were computed using the 11 points of equal-sized grids search of ρ from 0 to 1. For small sample adjusted SKAT and adjusted SKAT-O, the sample kurtosis was estimated from 10,000 bootstrapped phenotype sets. Three different total sample sizes ($n = 200, 500$, and $1,000$) were considered, with 10,000 simulated data sets for each sample size.

To investigate type I error rates in SKAT family tests when the α level is set at a level for exome-wide testing, we conducted simulations with slightly different settings. In order to reduce the computational

burden, we first generated 20,000 genotype sets of randomly selected regions, and then generated 500 phenotype sets for each genotype set. A total of 10^7 phenotypes were generated, and type I error rates were estimated by the proportion of p-values smaller than the given α level.

2.5.2 Power Simulations

To evaluate the power of the proposed unified tests and their small sample adjustments relative to the competing methods, we simulated datasets under the alternative model. As with the type I error simulations, we randomly selected $3kb$ regions from a broader $1Mb$ region, but we then randomly chose causal variants from the rare variants with true $MAF < 0.03$. The dichotomous phenotypes with 50% cases and 50% controls were simulated from

$$\text{logit}(\pi_i) = \gamma_0 + 0.5X_{i1} + 0.5X_{i2} + \beta_1g_{i1} + \cdots + \beta_sg_{is},$$

where (g_1, \dots, g_s) were selected causal variants. Covariates X_1 and X_2 followed the same distribution as in the type I error simulation, and γ_0 was chosen to create a disease prevalence of disease 0.01 under the null hypothesis.

To study the effects of varying proportions of variants being causal variants, we considered three different settings in which 10%, 20%, and 50% of the rare variants were causal variants. For each setting, we considered three different sign configurations of the non-zero β 's: all β_j s were positive, 80% of β_j s were positive, and 50% of β_j s were positive. We used $|\beta_j| = c|\log_{10}(p_j)|/2$, where p_j was the MAF of the j^{th} variant. When 10% of the rare variants were causal, $c = \log(7)$, which gives an odds ratio equal to 7 for a variant with $MAF = 0.01$. When 20% and 50% of the rare variants were causal variants, $c = \log(5)$ and $\log(2.5)$, respectively, so the powers would not be too close to one and we can distinguish the powers of different methods. For each setting, 1000 datasets were generated and the power was estimated as the proportion of p-values smaller than a given α level.

2.6 The NHLBI ALI Exome Sequencing Data

The Acute Lung Injury whole exome sequencing data were part of the LungGO of the NLBLI Exome Sequencing Project <http://www.nhlbi.nih.gov/resources/exome.htm>. We performed exome sequencing of 88 patients with Acute Lung Injury (ALI)²⁸ selected from the extremes of the severity spectrum. Patients with ALI and severe hypoxemia (Partial pressure of arterial oxygen/Fraction of inspired oxygen <200) were enrolled from the intensive care unit at the Massachusetts General Hospital. Those with very high or very low “ventilator-free days” (VFD), a composite variable measuring the degree of dependence on mechanical ventilation in the first 28 days of hospital admission,²⁹ were selected for sequencing. Exome sequencing was completed on 88 subjects (n=43 high severity ALI (VFD<2), n=45 low severity ALI (VFD< 24)) at the Northwest Genomics Center at the University of Washington.

To call SNP variants, the GATK tool of the Broad Institute was used,³⁰ and approximately 130,000 SNP variants on 17755 genes were identified. We subsequently filtered out variants with high missing rates (missing rate > 0.1) and low quality control scores using the GATK tool, i.e, keeping variants with Qual < 30, QD < 5, AB > 0.75 or SB > -0.10, % of missing < 10%. This gave a total of 106,736 variants.

For SKAT and the unified test (SKAT-O), we used all the variants. For the weighted burden test (W) and the counting based burden test (N), due to the very small sample size, we used MAF < 0.05 as the criterion to define rare variants to be included in the analysis. Any genes with fewer than four rare variants with (MAF < 0.05) were excluded from the analysis, as these genes have little information about association with the phenotype given the small sample size. A total of 6,488 genes remained for analysis. All six methods discussed in the simulation study were applied to the data. The first two principal components calculated using EIGENSOFT³¹ from all 106,736 variants were used as covariates to adjust possible population stratification.

3 Results

3.0.1 Type I Error Simulation Results

We evaluated the type I error rates of the existing and proposed methods using 10,000 simulated datasets. Table 1 shows that un-adjusted SKAT and the unified test (SKAT-O) had substantially less

than nominal empirical type I error rates. When sample sizes increase, empirical type I error rates became closer to the nominal α levels. We also note that the optimal unified test (SKAT-O) was less conservative than SKAT. In contrast, the small sample adjusted SKAT and optimal unified test (adjusted SKAT and adjusted SKAT-O) and the two burden tests (N and W) have well-controlled type I error rates.

To investigate the type I error rates with exome-wide α levels, we generated 10^7 data sets. The results are given in Table 2. Three different $\alpha = 10^{-3}, 10^{-4}$ and 2.5×10^{-6} levels were considered. Note that $\alpha = 2.5 \times 10^{-6}$ is Bonferroni-adjusted level $\alpha = 0.05$ when simultaneously testing 20,000 genes. In this simulation study, the two burden tests were excluded to save computational time. Table 2 clearly shows that the unadjusted SKAT and unified test (SKAT-O) had substantially deflated type I error rates for small sample sizes. The unified test (SKAT-O) was less conservative than SKAT, and had correct type I error control when the sample size was 1000. Both the proposed small sample adjusted SKAT and unified test (adjusted SKAT and adjusted SKAT-O) performed much better than their unadjusted counterparts in small samples. They controlled type I error rates accurately over all sample sizes and all significance levels. Overall, our type I error simulation results confirm empirically that the proposed small sample adjustment methods accurately control type I error rates.

3.0.2 Power Simulation Results

We compared the powers for the burden tests, SKAT and the unified test (SKAT-O) and their small sample adjustments, i.e., all the six methods considered in the type I error simulations. We first considered the scenario that all causal variants were deleterious variants, i.e. the effects of the causal variants were all in the same direction. Figure 1 reports that by properly controlling the type I error, the small sample adjusted SKAT (adjusted SKAT) was more powerful than SKAT in every configuration, and the power gap was large when the sample size was small or when the significance level was small. Power for the unified test (SKAT-O) and its small sample adjustment (adjusted SKAT-O) showed a similar pattern. Between the two burden tests, W was more powerful than N for these simulation configurations, suggesting that proper weighting can increase power.

When only 10% of the rare variants were causal, small sample adjusted SKAT (adjusted SKAT) was

the most powerful test. The burden tests had substantially lowest power, indicating that burden tests are not powerful in the presence of a large fraction of non-causal variants. When the proportion of causal rare variants increased, the burden tests performed better. When 50% of the rare variants were causal, the burden tests had a higher power than small sample adjusted SKAT.

The optimal unified tests (SKAT-O and adjusted SKAT-O) consistently performed very well in both settings above. They behave like SKAT when SKAT is more powerful than burden tests, and behave like burden tests when burden tests are more powerful than SKAT. Small sample adjusted optimal unified (adjusted SKAT-O) outperformed its unadjusted counterpart (SKAT-O), especially when sample sizes are small, e.g., $n=200$. When 20% of rare variants were causal, adjusted SKAT-O was the most powerful test.

We next performed simulations in which 20%/80% and 50%/50% of causal variants were protective/deleterious variants (Figure 2 and 3). The same odds ratio functions from above were used. Similar to the case when all causal variants were deleterious (Figure 1), small sample adjusted SKAT (adjusted SKAT) had higher power than SKAT, and small sample adjusted unified test (adjusted SKAT-O) had higher power than its unadjusted counterpart (SKAT-O). The presence of mixed protective and deleterious variants substantially reduced the powers of burden tests, since the effects of the causal variants canceled out. With 50%/50% of the causal variants being protective/deleterious, the powers of the burden tests were less than half those of SKAT and its small sample adjustment. The optimal unified test behaved similarly to SKAT but had better power than SKAT and the burden test when 50% of the rare variants were causal and 50%/50% of the causal variants being protective/deleterious. Small sample adjustment for both SKAT and the unified test improved the power. All tests had lower power relative to the situation in which all causal variants were deleterious (Figure 1). This is because for the given low prevalence, the presence of protective variants resulted in fewer causal variants selected into the case-control sample (Supplementary Table 1).

The power simulation results show that the optimal unified test (SKAT-O) is robust to the proportion of rare variants that are causal and to the directions of the causal variant effects (relative to the other tests); it performs very well in a wide range of situations; and it outperform SKAT and the burden tests. The proposed small sample adjustment increases the power by properly controlling for type

I error rate, especially when the sample size is small, or α is very small.

3.1 Analysis of the NHLBI Acute Lung Injury (ALI) Exome Sequence Data

We applied the six methods used in simulation studies (burden tests, SKAT, the unified test (SKAT-O), and their small sample adjustments) to analysis of the NHLBI ALI exome sequencing data of 88 subjects to identify genes associated with ALI severity. We restricted our analysis to the genes with at least four variants with MAFs < 0.05 . A total of 6,488 genes were analyzed (see the Method Section).

Figure 4 gives the QQ plots of the p-values calculated using all the six methods. Given the small sample size, no p-value achieved the Bonferroni adjusted genome-wide significance at $\alpha = 7.7 \times 10^{-6}$. The QQ plots of unadjusted SKAT and unified test (SKAT-O) were skewed downward, suggesting these tests were conservative. Interestingly, the QQ plots of the burden tests had a slightly anti-conservative pattern. The QQ plots of small sample adjusted SKAT and unified test (adjusted SKAT-O) were close to the 45 degree line, suggesting that the proposed small sample adjustment methods worked well and properly controlled type I error rates. There were eight genes with p-values $< 10^{-3}$ by the adjusted SKAT-O.

We next restricted our analysis to the functional variants that are missense, nonsense, and splicing sites variants. Similar to the first analysis, we only considered genes that have at least four functional variants with MAF < 0.05 . A total of 2,939 genes were used in analysis. The QQ plots of the six methods are given in Supplementary Figure 1. The patterns of these QQ plots are similar to those in Figure 4. There were five genes with p-values $< 10^{-3}$ by the adjusted SKAT-O. MYLK, a gene previously associated with susceptibility to ALI,^{32,33} was second most-significant in the adjusted SKAT analysis and 4th most significant in the adjusted SKAT-O analysis.

We compared the p-values obtained using small sample adjusted SKAT (adjusted SKAT) and the adjusted optimal unified test (adjusted SKAT-O) with those obtained using the burden test (W) (Supplementary Figure 2). These comparisons show that the p-values obtained using adjusted SKAT and W are quite different from each other, indicating that these two tests evaluate different aspects of association patterns. In contrast, the p-values obtained with adjusted SKAT-O were more highly correlated with those obtained with either adjusted SKAT or W as p-values declined, consistent with the expect-

tation that the optimal unified test uses the data to adaptively choose an optimal test to maximize power.

4 Discussions

In this paper, we present a unified rare variant test framework that includes both burden tests and the non-burden test SKAT as special cases. The proposed optimal unified test (SKAT-O) procedure uses the data to adaptively select the best linear combination of the burden test and SKAT to maximize test power. Similar to SKAT, the proposed SKAT-O is computationally efficient and easily adjusts for covariates such as age, gender and principal components for population stratifications. We show in simulation studies that SKAT and burden tests can both lose power when underlying assumptions are violated. However, the optimal unified test SKAT-O is optimal or near optimal in broad circumstances. In the SKAT package, we also provide power and sample size calculations using SKAT, SKAT-O and their small sample adjustments to help investigators design sequencing association studies.

In whole exome sequencing studies or whole genome sequencing studies, one would expect that some genes or genomic regions have a high proportion of causal variants with the same association direction, while other regions have many non-causal variants or causal variants with different association directions. Applying only either a burden test or SKAT would decrease the chance of detecting all of those genes. However, the use of SKAT-O can substantially increase the probability of detecting these genes.

Due to high sequencing costs, many of the existing whole exome sequencing studies have small sample sizes. As the second goal of this paper, we developed small sample adjustment methods to correct p-values for SKAT and SKAT-O to properly control the type I error rate and increase the power. Using extensive simulation studies and the NHLBI whole exomes from individuals who developed ALI, we demonstrated good performance of the proposed small sample adjustment methods both in terms of type I error control and power increase.

In this study, we only considered dichotomous traits. However, the application of SKAT-O to quantitative trait data is straightforward using equation (1) with a linear regression. Furthermore, we note that the small sample adjustment is not necessary for continuous traits, because the small sample

distributions of SKAT and SKAT-O are the same as their asymptotic distributions.

Recently several adaptive methods have been proposed to increase the power. For example, the VT test¹¹ tries to find the optimal MAF threshold of rare variants by varying the threshold, and EREC¹⁸ estimates a regression coefficient of each variant and uses them as the weight. Those approaches could improve the power compared to the burden tests. However, the VT test makes similar assumptions to the burden tests, i.e, requires majority of rare variants under the optimal threshold to be causal and have effects in the same direction. The EREC method requires estimation of regression coefficients, which are difficult to be estimated stably for rare variants. Addition of a stabilizing constant in EREC can reduce the power relative to asymptotic calculations and make the test behave like burden tests. Furthermore, these methods are computationally intensive when applied to large scale sequencing studies, e.g., whole exome(genome) sequencing studies, because they rely on a large number of permutation or bootstrap samples to compute p-values, and are difficult to control for covariates, such as population stratification. In contrast, SKAT-O and its small sample adjustment compute p-values efficiently and can be easily applied to whole exome(genome) sequencing studies.

We note that the proposed small sample adjustment methods are still computationally efficient even though we estimate the kurtosis using resampling. It only requires 10,000 bootstrap samples to accurately estimate the kurtosis, which is a substantially smaller computational burden compared to obtaining permutation or bootstrap p-values, which require 10^7 or 10^8 resampled phenotypes to accurately obtain p-values in the $10^{-5} \sim 10^{-6}$ ranges.

In simulation and real data analysis, we used a flexible beta weight to up-weight the influence of rarer variants. Similar results are obtained using logistic weight $w_j = \exp((a_1 - p_j)a_2) / \{1 + \exp((a_1 - p_j)a_2)\}$ for the ALI exome sequencing data (see Supplementary Figures 3 and 4). In addition to using a function of MAF of variants as weights, functional information can also be used to choose variants to be tested or to construct the weight. For example, only functional variants such as nonsense and missense variants can be used to test association, or functional information scores such as PolyPhen or SIFT scores^{21,22} can be used to construct a weight (an area under active study).

SUPPLEMENTAL DATA

Supplemental Data includes four figures one table can be found with this article online at <http://www.cell.com/AJHG>.

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Appendices

A. Mean and Variance of Q_S under the NULL hypothesis

Suppose $\tilde{\mathbf{y}} = \mathbf{D}^{-1/2}(\mathbf{y} - \boldsymbol{\pi})$, where $\mathbf{D} = \text{diag}[\pi_1(1 - \pi_1), \dots, \pi_n, (1 - \pi_n)]$. Then for all $j = 1, \dots, q$, $E[(\tilde{\mathbf{y}}' \mathbf{u}_j)^2 | \boldsymbol{\pi}, \mathbf{u}_j] = 1$, and

$$\begin{aligned} E[(\tilde{\mathbf{y}}' \mathbf{u}_j)^4 | \boldsymbol{\pi}, \mathbf{u}_j] &= \sum_{i=1}^n u_{ij}^4 E(\tilde{y}_i^4 | \boldsymbol{\pi}, \mathbf{u}_j) + 3 \sum_{i \neq k}^n u_{ij}^2 u_{ik}^2 E(\tilde{y}_i^2 | \boldsymbol{\pi}, \mathbf{u}_j) E(\tilde{y}_k^2 | \boldsymbol{\pi}, \mathbf{u}_j) \\ &= \sum_{i=1}^n u_{ij}^4 \pi_i (1 - \pi_i) (3\pi_i^2 - 3\pi_i + 1) / (\pi_i (1 - \pi_i))^2 + 3 \sum_{i \neq k}^n u_{ij}^2 u_{ik}^2, \end{aligned} \quad (\text{A.1})$$

where \tilde{y}_i is the i^{th} element of $\tilde{\mathbf{y}}$, and u_{ij} is the i^{th} element of \mathbf{u}_j . Therefore,

$$\text{var}[(\tilde{\mathbf{y}}' \mathbf{u}_j)^2 | \boldsymbol{\pi}, \mathbf{u}_j] = \sum_{i=1}^n u_{ij}^4 \pi_i (1 - \pi_i) (3\pi_i^2 - 3\pi_i + 1) / (\pi_i (1 - \pi_i))^2 + 3 \sum_{i \neq k}^n u_{ij}^2 u_{ik}^2 - 1.$$

Now we calculate the first two moments of Q given \mathbf{p} , \mathbf{U} and $\mathbf{\Lambda}$.

$$E(Q_S|\boldsymbol{\pi}, \mathbf{U}, \mathbf{\Lambda}) = E\left(\sum_{j=1}^q \lambda_j \tilde{\mathbf{y}}' \mathbf{u}_j \mathbf{u}_j' \tilde{\mathbf{y}} | \boldsymbol{\pi}, \mathbf{U}, \mathbf{\Lambda}\right) = \sum_{j=1}^q \lambda_j, \quad \text{and}$$

$$\begin{aligned} E(Q_S^2|\boldsymbol{\pi}, \mathbf{U}, \mathbf{\Lambda}) &= E\left[\left(\sum_{j=1}^q \lambda_j \tilde{\mathbf{y}}' \mathbf{u}_j \mathbf{u}_j' \tilde{\mathbf{y}}\right)^2 | \boldsymbol{\pi}, \mathbf{U}, \mathbf{\Lambda}\right] \\ &= \sum_{j=1}^q \lambda_j^2 E[(\tilde{\mathbf{y}}' \mathbf{u}_j)^4 | \boldsymbol{\pi}, \mathbf{U}, \mathbf{\Lambda}] \\ &\quad + \sum_{j \neq k} \lambda_j \lambda_k E\left[\left(\sum_{i_1, i_2} \tilde{y}_{i_1} \tilde{y}_{i_2} u_{i_1 j} u_{i_2 j}\right) \left(\sum_{l_1, l_2} \tilde{y}_{l_1} \tilde{y}_{l_2} u_{l_1 k} u_{l_2 k}\right) | \boldsymbol{\pi}, \mathbf{U}, \mathbf{\Lambda}\right] \end{aligned} \quad (\text{A.2})$$

Since $E(\tilde{y}_i|\boldsymbol{\pi}) = 0$, the elements in the second term in (A.2) can contribute to the overall sum only when 1) $i_1 = i_2 = l_1 = l_2$, 2) $i_1 = i_2$ and $l_1 = l_2$, 3) $i_1 = l_1$ and 4) $i_2 = l_2$ or $i_1 = l_2$ and $i_2 = l_1$. Therefore

$$\begin{aligned} &E\left[\left(\sum_{i_1, i_2} \tilde{y}_{i_1} \tilde{y}_{i_2} u_{i_1 j} u_{i_2 j}\right) \left(\sum_{l_1, l_2} \tilde{y}_{l_1} \tilde{y}_{l_2} u_{l_1 k} u_{l_2 k}\right) | \boldsymbol{\pi}, \mathbf{U}, \mathbf{\Lambda}\right] \\ &= \sum_{i=1}^n u_{ij}^2 u_{ik}^2 E(\tilde{y}_i^4 | \boldsymbol{\pi}) + \sum_{i_1 \neq i_2}^n u_{i_1 j}^2 u_{i_2 k}^2 E(a_{i_1}^2 | \boldsymbol{\pi}) E(a_{i_2}^2 | \mathbf{p}) + 2 \sum_{i_1 \neq i_2}^n u_{i_1 j} u_{i_2 j} u_{i_1 k} u_{i_2 k} E(a_{i_1}^2 | \boldsymbol{\pi}) E(a_{i_2}^2 | \boldsymbol{\pi}) \\ &= \sum_{i=1}^n u_{ij}^2 u_{ik}^2 \pi_i (1 - \pi_i) (3\pi_i^2 - 3\pi_i + 1) / (\pi_i (1 - \pi_i))^2 + \sum_{i_1 \neq i_2}^n u_{i_1 j}^2 u_{i_2 k}^2 + 2 \sum_{i_1 \neq i_2}^n u_{i_1 j} u_{i_2 j} u_{i_1 k} u_{i_2 k}. \end{aligned} \quad (\text{A.3})$$

We can calculate the second moment of Q_S by combining (A.2) and (A.3).

B. Null distribution of Small Sample SKAT-O

Define $\mathbf{Z} = \mathbf{D}^{-1/2} \mathbf{G} \mathbf{W}$ and $\bar{\mathbf{z}} = (\bar{z}_1, \dots, \bar{z}_n)'$, where $\bar{z}_i = \sum_{j=1}^m z_{ij} / m$. We further let $\mathbf{M} = \bar{\mathbf{z}} (\bar{\mathbf{z}}' \bar{\mathbf{z}})^{-1} \bar{\mathbf{z}}'$ and

$$\psi(\rho) = m^2 \rho \bar{\mathbf{z}}' \bar{\mathbf{z}} + \frac{1 - \rho}{\bar{\mathbf{z}}' \bar{\mathbf{z}}} \sum_{j=1}^m (\bar{\mathbf{z}}' \mathbf{z}_{\cdot j})^2,$$

where $\mathbf{z}_{.j}$ is the j^{th} column of \mathbf{Z} . Following the same argument in Lee *et al.*(2011),¹⁹ it can be shown that Q_ρ is equivalent as

$$(1 - \rho)\kappa_1 + \psi(\rho)\kappa_2, \quad (\text{A.4})$$

where

$$\kappa_1 = (1 - \rho)\tilde{\mathbf{y}}'(\mathbf{I} - \mathbf{M})\mathbf{Z}\mathbf{Z}'(\mathbf{I} - \mathbf{M})\tilde{\mathbf{y}} + 2(1 - \rho)\tilde{\mathbf{y}}'(\mathbf{I} - \mathbf{M})\mathbf{Z}\mathbf{Z}'\mathbf{M}\tilde{\mathbf{y}}$$

and

$$\kappa_2 = \tilde{\mathbf{y}}'\tilde{\mathbf{z}}\tilde{\mathbf{z}}'\tilde{\mathbf{y}}/\tilde{\mathbf{z}}'\tilde{\mathbf{z}}.$$

It can be shown that κ_2 asymptotically follows the χ_1^2 distribution, and κ_1 is asymptotically the same as

$$\sum_{k=1}^q \lambda_k \eta_k + \zeta,$$

where $\{\lambda_1, \dots, \lambda_q\}$ are non-zero eigenvalues of $\mathbf{Z}'(\mathbf{I} - \mathbf{M})\mathbf{Z}$, $\eta_k (k = 1, \dots, q)$ are i.i.d χ_1^2 random variables, and ζ satisfies the following conditions:

$$\begin{aligned} E(\zeta) &= 0, \quad Var(\zeta) = 4\text{trace}(\mathbf{Z}'\mathbf{M}\mathbf{Z}\mathbf{Z}'(\mathbf{I} - \mathbf{M})\mathbf{Z}), \\ Corr\left(\sum_{k=1}^q \lambda_k \eta_k, \zeta\right) &= 0, \quad \text{and} \quad Corr(\kappa_2, \zeta) = 0. \end{aligned}$$

We note that asymptotic p-values can be obtained by the one-dimensional integration. When the sample size is small, however, the asymptotic moments of κ_1 and κ_2 can be larger than small sample moments. Thus, we apply the same small sample adjustment procedure to null distributions of κ_1 and κ_2 . We first compute small sample variance and kurtosis of κ_1 and κ_2 , and apply the moment matching approximation to obtain the adjusted asymptotic distribution of them. To obtain a p-value, we apply the algorithm in Lee *et al.*(2011) with the adjusted null distribution κ_1 and κ_2 .

WEB RESOURCES

An implementation of SKAT and SKAT-O and their small sample adjustments as well as power/sample size calculations in the R language can be found at <http://www.hsph.harvard.edu/~xlin/software.html>.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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Table 1: Simulation Studies of type I error estimates of six different methods to test an association between randomly selected 3kb regions with dichotomous traits at $\alpha = 0.01$ and 0.05 . Each entry represents type I error rate estimates as the proportion of p-values smaller than α under the null hypothesis based on 10,000 simulated datasets.

α	N	W	SKAT	SKAT-O	adjusted SKAT	adjusted SKAT-O
SampleSize = 200						
0.05	5.72×10^{-2}	5.55×10^{-2}	3.36×10^{-2}	4.26×10^{-2}	5.34×10^{-2}	5.96×10^{-2}
0.01	1.28×10^{-2}	1.32×10^{-2}	2.90×10^{-3}	5.80×10^{-3}	9.75×10^{-3}	1.14×10^{-2}
SampleSize = 500						
0.05	5.53×10^{-2}	5.29×10^{-2}	4.59×10^{-2}	4.76×10^{-2}	5.26×10^{-2}	5.39×10^{-2}
0.01	1.09×10^{-2}	1.06×10^{-2}	7.95×10^{-3}	9.00×10^{-3}	1.13×10^{-2}	1.12×10^{-2}
SampleSize = 1000						
0.05	5.05×10^{-2}	5.14×10^{-2}	4.45×10^{-2}	4.71×10^{-2}	4.73×10^{-2}	4.91×10^{-2}
0.01	1.05×10^{-2}	1.14×10^{-2}	8.30×10^{-3}	1.00×10^{-2}	9.70×10^{-3}	1.11×10^{-2}

Table 2: Simulation studies of type I error estimates of four different methods to test an association between randomly selected 3kb regions with dichotomous traits at stringent α levels $\alpha = 10^{-3}, 10^{-4}$ and 2.5×10^{-6} . Each entry represents type I error rate estimates as the proportion of p-values smaller than α under the null hypothesis based on 10^7 simulated phenotypes.

α	SKAT	SKAT-O	adjusted SKAT	adjusted SKAT-O
SampleSize = 200				
10^{-3}	1.84×10^{-4}	5.03×10^{-4}	1.13×10^{-3}	1.24×10^{-3}
10^{-4}	5.30×10^{-6}	3.20×10^{-5}	1.01×10^{-4}	1.04×10^{-4}
2.5×10^{-6}	1.00×10^{-7}	3.00×10^{-7}	3.20×10^{-6}	2.50×10^{-6}
SampleSize = 500				
10^{-3}	5.17×10^{-4}	8.14×10^{-4}	1.12×10^{-3}	1.16×10^{-3}
10^{-4}	2.95×10^{-5}	7.13×10^{-5}	1.14×10^{-4}	1.12×10^{-4}
2.5×10^{-6}	1.00×10^{-7}	1.00×10^{-6}	2.50×10^{-6}	2.30×10^{-6}
SampleSize = 1000				
10^{-3}	7.22×10^{-4}	1.00×10^{-3}	1.09×10^{-3}	1.12×10^{-3}
10^{-4}	5.59×10^{-5}	1.02×10^{-4}	1.22×10^{-4}	1.19×10^{-4}
2.5×10^{-6}	1.00×10^{-6}	2.80×10^{-6}	3.20×10^{-6}	3.10×10^{-6}

Figure 1: Empirical power of the six methods for randomly selected 3kb regions and all causal variants were deleterious. From top to bottom, the plots consider the significance levels 0.01, 10^{-3} , and 2.5×10^{-6} , respectively. From left to right, the plots consider settings in which 10% of rare variants were causal, 20% of rare variants were causal, 50% of rare variants were causal. For causal variants, we assumed $|\beta_j| = c|\log_{10}(p_j)|/2$, where p_j was the MAF of the j^{th} variant. Different c was used for the three panels from left to right: $c = \log(7), \log(5), \log(2.5)$ for the percentage of causal variants being 10%, 20%, and 50% respectively. Hence the powers between the three panels from left to right are not comparable. Total sample sizes considered were 200, 500, and 1000, with half being cases in case-control studies.

Figure 2: Empirical power of the six methods for randomly selected 3kb regions and 20%/80% of causal variants were protective/deterious. From top to bottom, the plots consider the significance levels 0.01, 10^{-3} , and 2.5×10^{-6} , respectively. From left to right, the plots consider settings in which 10% of rare variants were causal, 20% of rare variants were causal, 50% of rare variants were causal. For causal variants, we assumed $|\beta_j| = c|\log_{10}(p_j)|/2$, where p_j was the MAF of the j^{th} variant. Different c was used for the three panels from the left to the right: $c = \log(7), \log(5), \log(2.5)$ for the percentage of causal variants being 10%, 20%, and 50% respectively. Hence the powers between the three panels from left to right are not comparable. Total sample sizes considered were 200, 500, and 1000, with half being cases in case-control studies.

Figure 3: Empirical power of the six methods for randomly selected 3kb regions and 50%/50% of causal variants were protective/deterious. From top to bottom, the plots consider the significance levels 0.01, 10^{-3} , and 2.5×10^{-6} , respectively. From left to right, the plots consider settings in which 10% of rare variants were causal, 20% of rare variants were causal, 50% of rare variants were causal. For causal variants, we assumed $|\beta_j| = c|\log_{10}(p_j)|/2$, where p_j was the MAF of the j^{th} variant. Different c was used for the three panels from left to right: $c = \log(7), \log(5), \log(2.5)$ for the percentage of causal variants being 10%, 20%, and 50%. Hence the powers between the three panels from left to right are not comparable. Total sample sizes considered were 200, 500, and 1000, with half being cases in case-control studies.

Figure 4: $-\log_{10}$ QQ plots of observed vs. expected p-values for ALI exome sequence data for the six methods: burden tests (N,W), SKAT, SKAT-O, adjusted SKAT, adjusted SKAT-O. X-axis represents $-\log_{10}$ expected p-values, and Y-axis represents $-\log_{10}$ observed p-values. A total of 6,488 genes with at least four rare variants were tested for associations with ALI severity.

Figure1

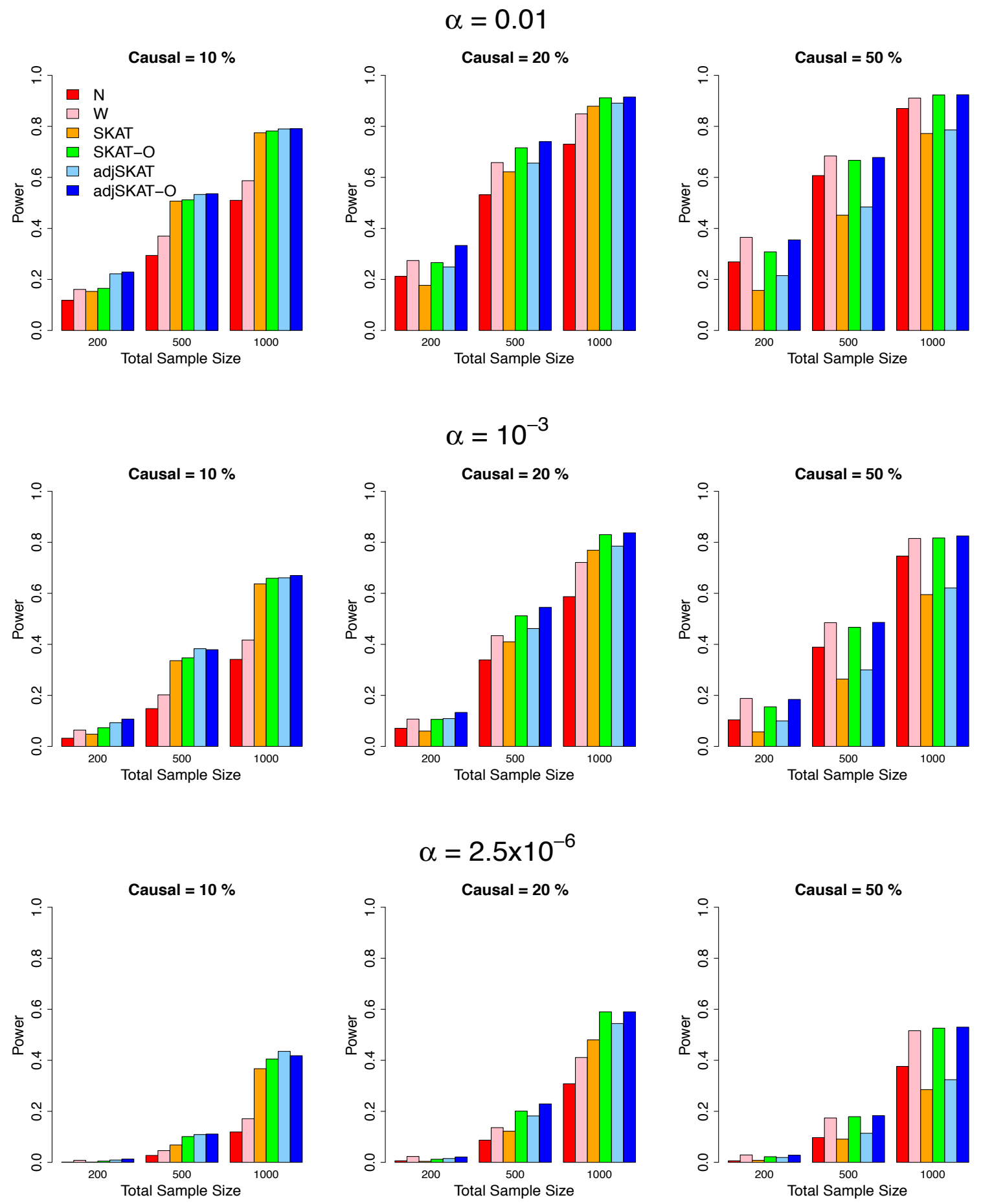


Figure2

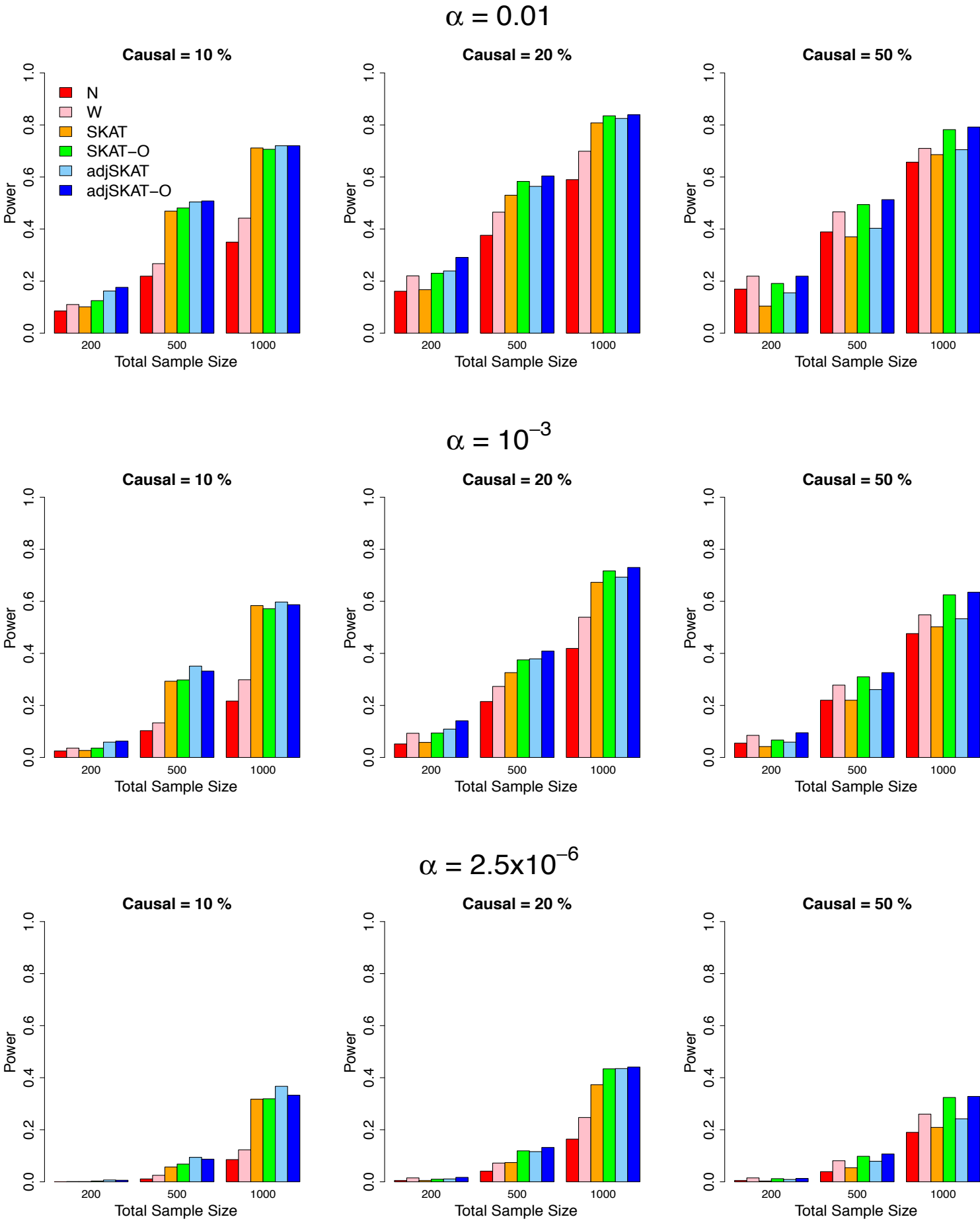


Figure3

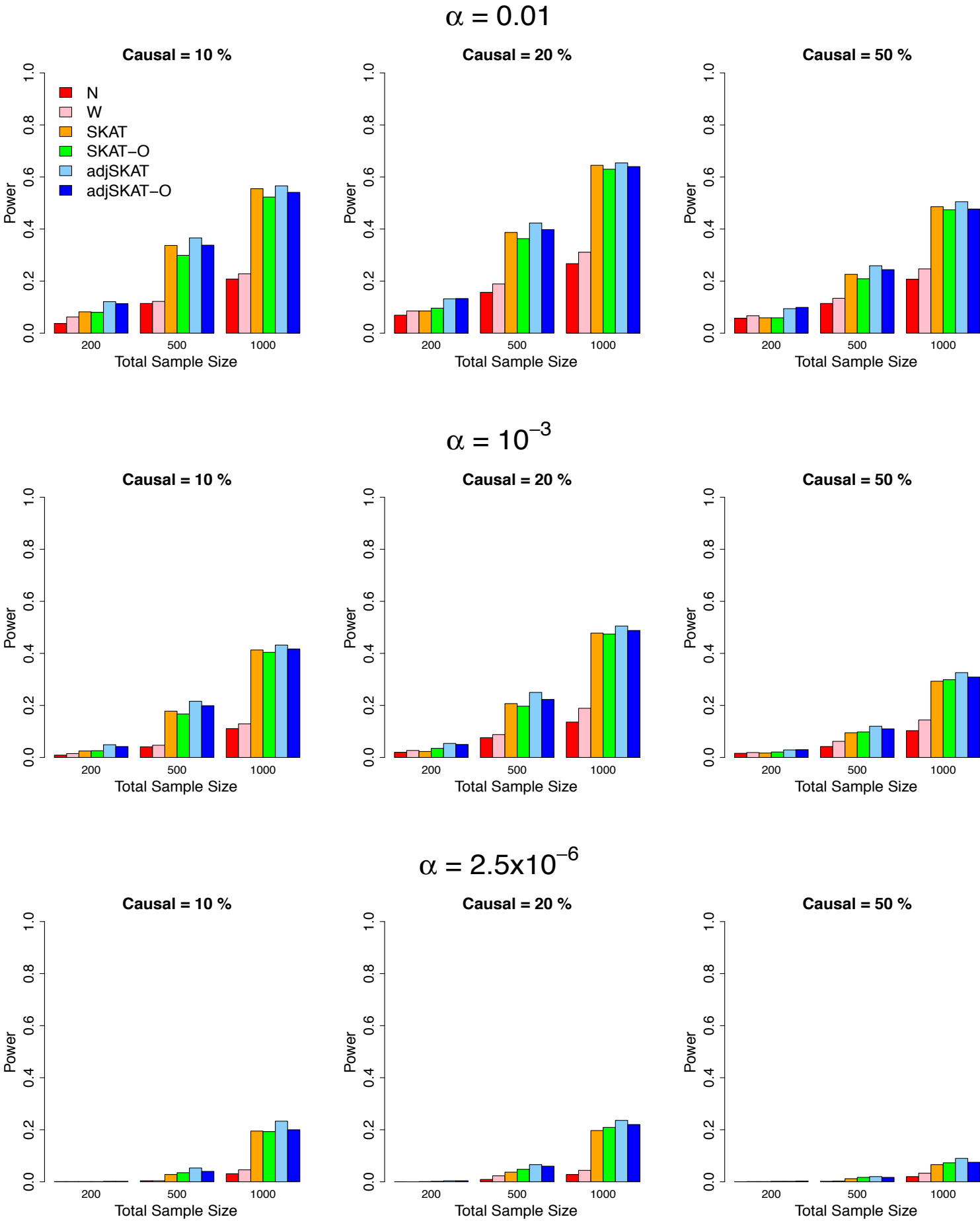
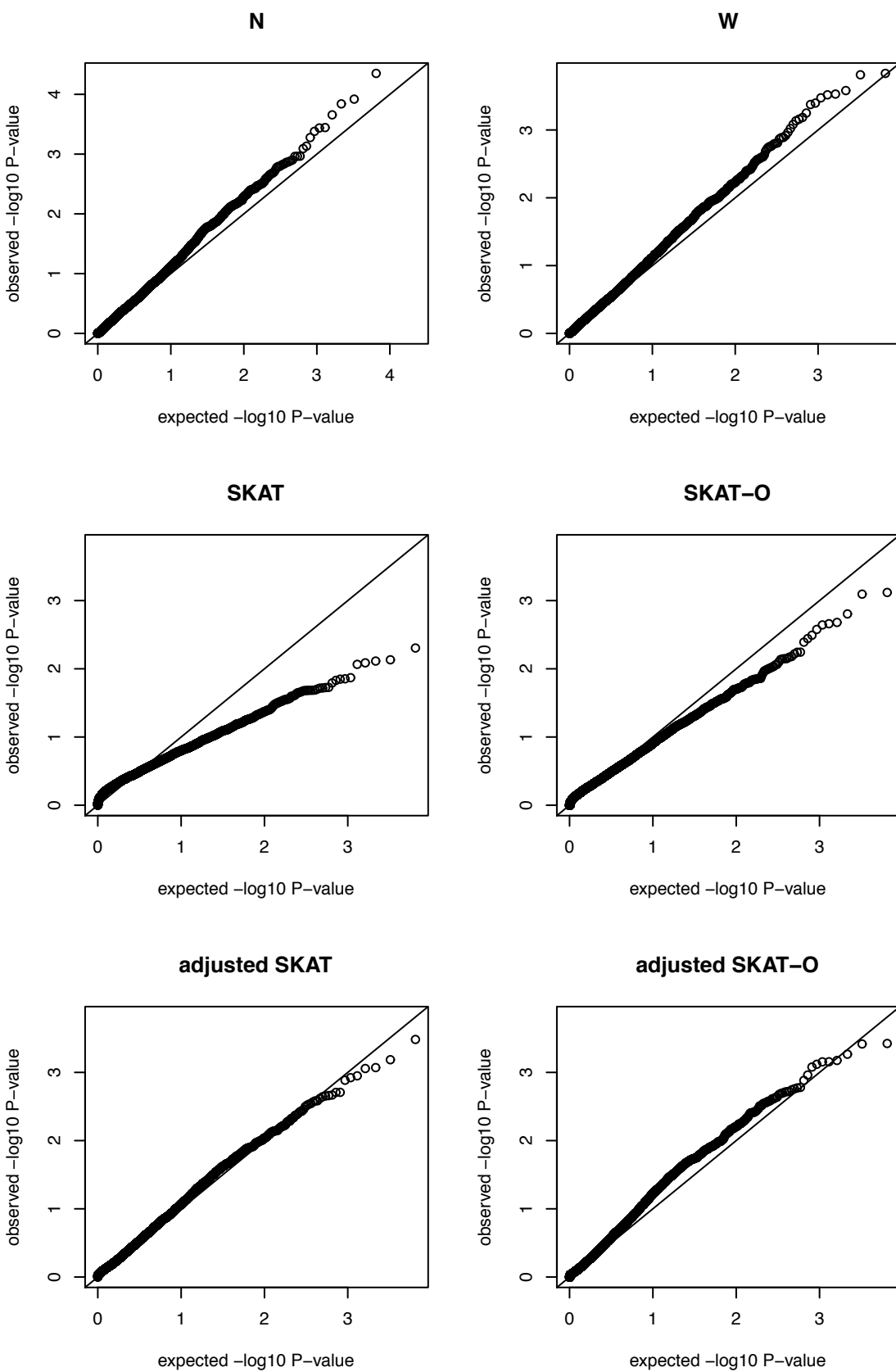


Figure4



Supplemental Data

[Click here to download Supplemental Movies and Spreadsheets: Supplement_Data.pdf](#)