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On set-based association tests: Insights from a regression using summary statistics

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Abstract: Motivated by, but not limited to, association analyses of multiple genetic variants, we propose here a summary statistics-based regression framework. The proposed method requires only variant-specific summary statistics, and it unifies earlier methods based on individual-level data as special cases. The resulting score test statistic, derived from a linear mixed-effect regression model, inherently transforms the variant-specific statistics using the precision matrix to improve power for detecting sparse alternatives. Furthermore, the proposed method can incorporate additional variant-specific information with ease, facilitating omic-data integration. We study the asymptotic properties of the proposed tests under the null and alternatives, and we investigate efficient *P*-value calculation in finite samples. Finally, we provide supporting empirical evidence from extensive simulation studies and two applications. *The Canadian Journal of Statistics* 00: 000–000; 2020 © 2020 Statistical Society of Canada

Résumé: Motivées notamment par les analyses d'association entre plusieurs variantes génétiques, les auteures proposent un cadre de régression basé sur les statistiques sommaires. La méthode proposée ne requiert que des statistiques sommaires pour chacune des variantes, et elle unifie les méthodes précédentes basées sur les données individuelles comme cas particuliers. La statistique score de test qui en résulte est dérivée d'un modèle de régression linéaire à effets mixtes et transforme les statistiques spécifiques aux variantes à l'aide d'une matrice de précision pour améliorer la puissance de détection des alternatives éparses. La méthode proposée peut également incorporer aisément des informations additionnelles spécifiques aux variantes, ce qui facilite l'intégration des données omiques. Les auteures étudient les propriétés asymptotiques des tests proposés sous les hypothèses nulle et alternative, et elles investiguent le calcul efficace de p-values pour les échantillons finis. Elles illustrent et supportent leur méthode empiriquement par de vastes études de simulation et deux applications. *La revue canadienne de statistique* 00: 000–000; 2020 © 2020 Société statistique du Canada

1. INTRODUCTION

Set-based joint analyses of multiple variables are increasingly important in many current scientific studies. For example, in modern genome-wide association studies (GWAS), one might be interested in jointly analyzing multiple (rare) genetic variants influencing a complex, and

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heritable trait (also known as gene- or pathway-based association studies), identifying one genetic variant influencing multiple traits (also known as pleiotropy studies), or combining evidence from multiple studies as in the classical meta-analyses.

Without loss of generality, let us focus on set-based analyses of multiple rare genetic variants. In this setting, myriad statistical tests have been proposed, and they fit into three general categories (e.g., Derkach, Lawless & Sun, 2014; Lee et al., 2014): the linear or burden tests (e.g., Madsen & Browning, 2009), the quadratic or variance-component tests (e.g., Pan, 2009; Wu et al., 2011), and the hybrid tests combining evidence from the linear and quadratic tests (e.g., Lee, Wu & Lin, 2012; Derkach, Lawless & Sun, 2013).

These earlier methods have been studied extensively, but they can be improved in several aspects. First, these methods may not perform well in the sparse-signal setting where only a small proportion of the variants are truly associated (Xu et al., 2016). Second, individual/subject-level data may not be available in practice, so it is useful to develop summary statistics-based association tests, and ideally the tests can also incorporate additional information available for each variant. Finally, earlier works have shown that the performance of a simple minimum-P value approach (Derkach, Lawless & Sun, 2013) is comparable with that of the optimal sequence kernel association test, SKAT-O (Lee, Wu & Lin, 2012). This suggests that a grid search for the "optimal" weighting factor may not be necessary, and it is helpful to develop new robust hybrid test statistics with model-driven weights.

To this end, we propose a flexible and unifying linear mixed-effect regression model that requires only variant-specific summary statistics, and we show that earlier methods based on individual-level data are special cases of the proposed testing framework. The set-based association test statistic derived from the regression model inherently transforms the variant-specific summary statistics using the precision matrix to improve power for detecting sparse alternatives; see Fan, Jin & Yao (2013) and Cai, Liu & Xia (2014) for the use of precision matrix in other high-dimension analytical settings. Furthermore, the proposed method can incorporate additional variant-specific information as a covariate, e.g., the functional importance of the variants to be analyzed (Ionita-Laza et al., 2016). Through simulation and application studies, we show that the proposed method can have substantial power gain when the included covariate contains useful information, while power loss is minimal when the covariate is uninformative.

Although the proposed set-based regression approach is motivated by joint analyses of multiple rare genetic variants, the method can be used in other settings, for example, meta-analyses of multiple studies (Han & Eskin, 2011), data integration of GWAS and gene-expression data (Gamazon et al., 2015), pleiotropy association studies of multiple phenotypes (Liu & Lin, 2018b), as well as polygenic risk score (PRS) analyses of multiple common variants (Purcell et al., 2009). We will discuss later the differences and connections between the proposed method and these earlier works.

The remainder of the article is organized as follows. Section 2 reviews existing association methods for jointly analyzing a set of rare genetic variants based on individual-level data. Section 3 first outlines the proposed regression framework based on summary statistics, from which we derive a catalogue of association test statistics from fixed-, random-, or mixed-effect models. We then demonstrate the analytical equivalence between some of the newly proposed statistics and the existing ones for rare variants analyses. This section also investigates efficient P-value calculation in finite samples, studies the asymptotic properties of the proposed tests, and considers covariate adjustments. Section 4 provides supporting empirical evidence from extensive simulation studies, and Section 5 presents results from two application studies. Section 6 concludes with remarks and discussion, and the Supplementary Material provides theoretical proofs and additional numerical results.

2. EXISTING ASSOCIATION TESTS FOR JOINTLY ANALYZING A SET OF RARE GENETIC VARIANTS

2.1. Regression Set-up Using Individual-level Data

Consider a sample of n independent individuals and a set of J genetic variants of interest; let $\boldsymbol{y} = (y_1, \ldots, y_n)^T$ denote the phenotype variable and $\boldsymbol{G}$ be a $n \times J$ matrix for the corresponding genotypes with elements G_{ij}, $i = 1, \ldots, n$ and $j = 1, \ldots, J$, $\boldsymbol{G}_i = (G_{i1}, G_{i2}, \ldots, G_{iJ})^T$ and $\boldsymbol{G}_j = (G_{1j}, G_{2j}, \ldots, G_{nj})^T$. Assume that y_i given $\boldsymbol{G}_i$ follows an exponential family distribution with mean μ_i, and consider using the canonical link function $g(\cdot)$ and

$$g(\mu_i) = g\left(E(y_i|\boldsymbol{G}_i)\right) = \beta_0 + \beta_1 G_{i1} + \cdots + \beta_J G_{iJ}, \tag{1}$$

to model the phenotype–genotype association, where β_j is the regression coefficient for genetic variant j. Individual-level covariates information such as age and sex, if available, should be added to the model but are omitted for the moment for notation simplicity.

We are interested in testing the hypothesis that

$$H_0 : \boldsymbol{\beta} = (\beta_1, \ldots, \beta_J)^T = \boldsymbol{0} \text{ v.s. } H_1 : \beta_j \neq 0, \text{ for any } j, j = 1, \ldots, J.$$

The corresponding score vector is

$$\boldsymbol{s} = (s_1, \ldots, s_J)^T = \boldsymbol{G}^T(\boldsymbol{y} - \bar{\mu}_y \boldsymbol{1_n}),$$

where $\boldsymbol{1_n}$ is a $n \times 1$ unit vector, $\bar{\mu}_y = \frac{1}{n}\sum_{i=1}^n y_i$ and $s_j = \sum_{i=1}^n (y_i - \bar{\mu}_y) G_{ij}$ capturing the linear relationship between phenotype $\boldsymbol{y}$ and genotype $\boldsymbol{G}_j$. The variance-covariance matrix of $\boldsymbol{s}$ is

$$\boldsymbol{\Sigma_0} = g_1^{-1}(\bar{\mu}_y)\boldsymbol{G}^T(\boldsymbol{I_n} - \boldsymbol{1_n}\boldsymbol{1_n}^T/n)\boldsymbol{G}, \tag{2}$$

where $g_1(\cdot)$ denotes the first derivative of the link function $g(\cdot)$, and $\boldsymbol{I_n}$ is a identity matrix of size n.

2.2. Existing Methods Based on the Score Vector $\boldsymbol{s}$

Although it may not be obvious, most test statistics developed for rare variants analyses are functions of $\boldsymbol{s}$. For example, the original burden test (Madsen & Browning, 2009) first constructs a "super-allele," $G^* = \sum_{j=1}^J w_j G_{ij}$, where $\boldsymbol{w} = (w_1, w_2, \ldots, w_J)^T$ are pre-specified weights often associated with the minor allele frequency (MAF) of the variants. The burden test then considers regression model, $g(\mu_i) = \beta_0^* + \beta^* G^*$ and test $H_0 : \beta^* = 0$, v.s. $H_1 : \beta^* \neq 0$. However, it is not difficult to show that the score test statistic derived from the regression using G^* is proportional to

$$T_1 = (\boldsymbol{w}^T\boldsymbol{s})^2,$$

where under the null of no association $\left(\boldsymbol{w'\Sigma_0 w}\right)^{-1} T_1$ follows a central chi-square distribution with 1 degrees of freedom (d.f.), χ_1^2, for a fixed J but assuming that $\boldsymbol{s}$ is multivariate normal asymptotically with respect to n (Derkach, Lawless & Sun, 2014).

This T_1 test is also termed as the sum test by Pan (2009), and it belongs to the linear class of tests (Derkach, Lawless & Sun, 2014). Because T_1 is based on the weighted average of s_j, and s_j can be positive or negative depending on the direction of effect (i.e., sign of β_j in model (1)), T_1 is only powerful when a large proportion of variants are causal *and* effects are in the same direction.

Variance-component tests, such as SSU (Pan, 2009) and SKAT (Wu et al., 2011), are alternatives that belong to the quadratic class of tests; see Table 1 of Derkach, Lawless & Sun (2014) for a summary. Again, although most of these tests started with regression model (1), they can be formulated as

$$T_2 = \boldsymbol{s}^T \boldsymbol{A} \boldsymbol{s},$$

where $\boldsymbol{A} = \text{diag}\{w_1^2, \ldots, w_J^2\}$ and w_j depends on the MAF of variant $j, j = 1, \ldots, J$. Under the null and the multivariate normality assumption for $\boldsymbol{s}$, T_2 follows a weighted sum of χ_1^2 distribution for a fixed J (Derkach, Lawless & Sun, 2014). T_2 is robust to heterogeneous effect directions, but it is less powerful than T_1 when most variants are causal and with the same direction of effects.

Since the true genetic model is unknown in practice, hybrid tests combining T_1 and T_2 have been proposed. For example, SKAT-O of Lee, Wu & Lin (2012) considers

$$Q_\rho = \rho(\boldsymbol{w}^T \boldsymbol{s})^2 + (1-\rho)\boldsymbol{s}^T \boldsymbol{A} \boldsymbol{s} = \rho T_1 + (1-\rho) T_2.$$

It then performs a grid search for the "optimal" ρ, $0 = \rho_1 < \rho_2 < \cdots < \rho_m = 1$. Letting p_ρ be the P-value corresponding to each Q_ρ, the final SKAT-O test statistic is

$$T_{skato} = \min\{p_{\rho_1}, \ldots, p_{\rho_m}\}.$$

The asymptotic P-value of T_{skato} can be calculated with one-dimensional numerical integration (Lee, Wu & Lin, 2012).

Instead of considering data-driven "optimal" ρ and adjusting for the inherent selection bias, Derkach, Lawless & Sun (2013) proposed two simpler yet competitive hybrid test statistics, T_{Fisher} and T_{Minp}. Letting p_{T_1} and p_{T_2} be the P-values corresponding to T_1 and T_2, respectively, the Fisher and Minp statistics take the form of

$$T_{Fisher} = -2\log(p_{T_1}) - 2\log(p_{T_2}) \text{ and } T_{Minp} = \min\{p_{T_1}, p_{T_2}\}.$$

Under the null of no association, p_{T_1} and p_{T_2} are asymptotically independent of each other; thus, T_{Fisher} is χ_4^2 distributed and T_{Minp} is $Beta(1,2)$ distributed.

Derkach, Lawless & Sun (2013) have also shown that T_{Minp} and T_{skato} perform similarly, and they are slightly more powerful than T_{Fisher} when T_1 has no power (e.g., is less than 20%). On the other hand, when both T_1 and T_2 have some power, T_{Fisher} is more powerful than T_{Minp} and T_{skato}. However, we expect all three hybrid tests to have diminished power of detecting sparse alternatives (Donoho & Jin, 2004; Barnett, Mukherjee & Lin, 2017) when only a small proportion of the variants in a set are truly associated.

To improve performance, we first note that if variants are correlated with each other, we can consider for example $\boldsymbol{\Sigma}_0^{-1}\boldsymbol{s}$ instead of $\boldsymbol{s}$ to construct a more powerful test for detecting sparse alternatives. Second, it is clear now that we only need variant-specific summary statistics $\boldsymbol{s} = (s_1, \ldots, s_J)^T$ to jointly analyze the J variants of interest, provided $\boldsymbol{\Sigma_0}$ can be accurately estimated. Furthermore, the fact of T_{Minp} and T_{skato} having similar performance suggests that a grid search for ρ may not be necessary, and an easy-to-compute yet theoretically justified "optimal" ρ may exist. Lastly, when additional variant-specific information z_j (e.g., variant j being non-synonymous or not) is available, we can improve power by incorporating $\boldsymbol{z} = (z_1, \ldots, z_J)^T$. Intuitively we can achieve this by modifying w_j to incorporate z_j in addition to the MAF, but a less ad hoc approach is desirable. In the section, we develop a flexible and unifying regression framework that (i) requires only s_j and $\boldsymbol{\Sigma_0}$, (ii) yields T_1 and T_2 as special cases and a hybrid statistic similar to T_{skato}, and (iii) provides new test statistics that incorporate the precision matrix $\boldsymbol{\Sigma_0}^{-1}$ and account for covariate information $\boldsymbol{z}$.

3. A REGRESSION FRAMEWORK BASED ON SUMMARY STATISTICS

Here we assume that $\boldsymbol{s} = (s_1, \ldots, s_J)^T$ is available, summarizing the association relationship between the phenotype of interest and a set of J genetic variants as detailed in Section 2. We also assume that $\boldsymbol{\Sigma_0}$ is known or estimated accurately from a reference panel (Cheng et al., 2020; Fan, Liao & Liu, 2016). Let $\boldsymbol{z} = (z_1, \ldots, z_J)^T$ represent variant-specific information available.

3.1. Fixed-effect and Random-effect Models

We first consider a fixed-effect (FE) model that models the common effect μ present among $s_j, j = 1, \ldots, J$,

$$\boldsymbol{s} = \mu \boldsymbol{w} + \boldsymbol{\varepsilon}, \tag{3}$$

where $\boldsymbol{w} = (w_1, \ldots, w_J)^T$, $\boldsymbol{\varepsilon} = (\varepsilon_1, \ldots, \varepsilon_J)^T$, and $\boldsymbol{\varepsilon} \sim N(\boldsymbol{0}, \boldsymbol{\Sigma_0})$. Based on this model, we test

$$H_0 : \mu = 0 \text{ v.s. } H_1 : \mu \neq 0, \tag{4}$$

and the corresponding score test statistic is

$$T_{FE} = \left(\boldsymbol{w}^T \boldsymbol{\Sigma_0}^{-1} \boldsymbol{w}\right)^{-1} (\boldsymbol{w}^T \boldsymbol{\Sigma_0}^{-1} \boldsymbol{s})^2. \tag{5}$$

If we let $\boldsymbol{w}$ in T_{FE} be $\boldsymbol{\Sigma_0 w}$, the analytical equivalence between T_{FE} and T_1 in Section 2 is easy to show, because $T_1 \propto \left(\boldsymbol{w}^T \boldsymbol{\Sigma_0 w}\right)^{-1} (\boldsymbol{w}^T \boldsymbol{s})^2$ (Table 1).

Alternatively, we can consider the following random-effect (RE) model,

$$\boldsymbol{s} = \boldsymbol{\eta} + \boldsymbol{\varepsilon}, \tag{6}$$

where $\boldsymbol{\eta} \sim N(\boldsymbol{0}, \tau^2 \boldsymbol{R})$, $\boldsymbol{R}$ is a $J \times J$ pre-defined positive or semi-definite symmetric matrix, and $\boldsymbol{\varepsilon}$ is as defined above. If we test

$$H_0 : \tau^2 = 0 \text{ v.s. } H_1 : \tau^2 \neq 0, \tag{7}$$

the corresponding score test statistic is

$$T_{RE} = \left(2tr\left(\boldsymbol{\Sigma_0}^{-1} \boldsymbol{R}\right)^2\right)^{-1/2} \left(\boldsymbol{s}^T \boldsymbol{\Sigma_0}^{-1} \boldsymbol{R} \boldsymbol{\Sigma_0}^{-1} \boldsymbol{s} - tr(\boldsymbol{\Sigma_0}^{-1} \boldsymbol{R})\right) = c_2^{-1/2}(Q(\boldsymbol{s}) - c_1), \tag{8}$$

where $Q(\boldsymbol{s}) = \boldsymbol{s}^T \boldsymbol{\Sigma_0}^{-1} \boldsymbol{R} \boldsymbol{\Sigma_0}^{-1} \boldsymbol{s}$, and $c_1 = tr(\boldsymbol{\Sigma_0}^{-1} \boldsymbol{R})$ and $c_2 = 2tr(\boldsymbol{\Sigma_0}^{-1} \boldsymbol{R})^2$ are respectively the mean and variance of $Q(\boldsymbol{s})$. If we let $\boldsymbol{R} = \boldsymbol{\Sigma_0 A \Sigma_0}$, the analytical equivalence between T_{RE} and $T_2 = \boldsymbol{s}^T \boldsymbol{A s}$ is also apparent because $T_{RE} \propto Q(\boldsymbol{s})$ (Table 1). In addition, if we let $\boldsymbol{R} = \boldsymbol{\Sigma_0 W R_\rho W \Sigma_0}$, where $\boldsymbol{W} = \text{diag}\{w_j\}$ and $\boldsymbol{R}_\rho = \rho \boldsymbol{1_J 1_J}^T + (1 - \rho) \boldsymbol{I_J}$, then $Q(\boldsymbol{s}) = \rho T_1 + (1 - \rho) T_2 = Q_\rho$, the key element of T_{skato}.

3.2. Mixed-effect Model

The fixed-effect model (3) captures the common underlying effect, while the random-effect model (6) accounts for potential effect heterogeneity. A logical next step is to consider a mixed-effect (ME) modelling framework that includes models (3) and (6) as special cases,

$$\boldsymbol{s} = \mu \boldsymbol{w} + \boldsymbol{\eta} + \boldsymbol{\varepsilon}, \tag{9}$$

where $\boldsymbol{w}$, $\boldsymbol{\eta}$, and $\boldsymbol{\varepsilon}$ are defined as before. If we test

$$H_0 : \mu = 0, \tau^2 = 0 \text{ v.s. } H_1 : \mu \neq 0 \text{ or } \tau^2 \neq 0, \tag{10}$$

the corresponding score vector is $\left(\boldsymbol{w}^T\boldsymbol{\Sigma_0}^{-1}\boldsymbol{s},\ \frac{1}{2}\boldsymbol{s}^T\boldsymbol{\Sigma_0}^{-1}\boldsymbol{R}\boldsymbol{\Sigma_0}^{-1}\boldsymbol{s} - \frac{1}{2}tr(\boldsymbol{\Sigma_0}^{-1}\boldsymbol{R})\right)^T$, and the test statistic is

$$T_{ME} = \left(\boldsymbol{w}^T\boldsymbol{\Sigma_0}^{-1}\boldsymbol{w}\right)^{-1}(\boldsymbol{w}^T\boldsymbol{\Sigma_0}^{-1}\boldsymbol{s})^2 + c_2^{-1}\left(\boldsymbol{s}^T\boldsymbol{\Sigma_0}^{-1}\boldsymbol{R}\boldsymbol{\Sigma_0}^{-1}\boldsymbol{s} - c_1\right)^2, \tag{11}$$

where $c_1 = tr(\boldsymbol{\Sigma_0}^{-1}\boldsymbol{R})$ and $c_2 = 2tr(\boldsymbol{\Sigma_0}^{-1}\boldsymbol{R})^2$, the same as for T_{RE}.

Note that T_{ME} uses $\boldsymbol{\Sigma}_0^{-1}\boldsymbol{s}$ to account for the correlation between the tested variants. Transforming $\boldsymbol{s}$ by the precision matrix has been considered previously in other settings. For example, Cai, Liu & Xia (2014) showed that the transformation can improve the power of a two-sample high-dimensional means test for detecting sparse alternatives in the presence of high correlation.

Following the construction of T_{ME}, intuitively we can also consider the following weighted average of $T_1 = (\boldsymbol{w}^T\boldsymbol{s})^2$ and $T_2 = \boldsymbol{s}^T\boldsymbol{A}\boldsymbol{s}$ (and $\boldsymbol{A} = \boldsymbol{R}$),

$$T_{12} = \left(\boldsymbol{w}^T\boldsymbol{\Sigma_0}\boldsymbol{w}\right)^{-1}(\boldsymbol{w}^T\boldsymbol{s})^2 + \check{c}_2^{-1}\left(\boldsymbol{s}^T\boldsymbol{R}\boldsymbol{s} - \check{c}_1\right)^2, \tag{12}$$

where $\check{c}_1 = tr(\boldsymbol{\Sigma_0}\boldsymbol{R})$ and $\check{c}_2 = 2tr(\boldsymbol{\Sigma_0}\boldsymbol{R})^2$ are respectively the mean and variance of T_2 under the H_0 of (10). The connection between T_{12} with T_{skato} is also immediate. However, there are two key differences. First, given a ρ, T_{skato} relies on $Q_\rho = \rho T_1 + (1-\rho)T_2$. In contrast, T_{12} combines T_1 and the *square* of *centralized* T_2 (not T_2 itself). Second, T_{skato} searches for the "optimal" ρ that minimizes the P-value associated with Q_ρ and then adjusts for selection bias. In contrast, T_{12} uses the variances of T_1 and T_2 as weights.

3.3. Covariate Adjustment

Given $\boldsymbol{z} = (z_1, \ldots, z_J)^T$, one could consider directly modifying $\boldsymbol{w}$ to reflect the additional information available for the J variants of interest, but a principled approach is lacking. The proposed regression framework, however, can naturally incorporate $\boldsymbol{z}$ as a covariate into the regression,

$$\boldsymbol{s} = \mu\boldsymbol{w} + \theta\boldsymbol{z} + \boldsymbol{\eta} + \boldsymbol{\varepsilon}, \tag{13}$$

where $\boldsymbol{w}$, $\boldsymbol{\eta}$, and $\boldsymbol{\varepsilon}$ are defined as before. If we are interested in testing,

$$H_0 : \mu = 0, \theta = 0, \tau^2 = 0 \text{ v.s. } H_1 : \mu \neq 0 \text{ or } \theta \neq 0 \text{ or } \tau^2 \neq 0,$$

the corresponding score test statistic is

$$T_{ME,\,cov} = \boldsymbol{s}^T\boldsymbol{\Sigma_0}^{-1}\boldsymbol{u}(\boldsymbol{u}^T\boldsymbol{\Sigma_0}^{-1}\boldsymbol{u})^{-1}\boldsymbol{u}^T\boldsymbol{\Sigma_0}^{-1}\boldsymbol{s} + c_2^{-1}\left(\boldsymbol{s}^T\boldsymbol{\Sigma_0}^{-1}\boldsymbol{R}\boldsymbol{\Sigma_0}^{-1}\boldsymbol{s} - c_1\right)^2, \tag{14}$$

where $\boldsymbol{u} = (\boldsymbol{w}, \boldsymbol{z})$, and c_1 and c_2 are defined as before. Table 1 summarizes all the tests discussed so far.

3.4. Asymptotic Distributions of the Proposed Test Statistics

The asymptotic distributions of the existing tests, T_1, T_2, T_{skato}, T_{Fisher}, and T_{Minp}, have been previously established. Here, we establish the asymptotic distribution of T_{ME} and T_{12} when $J \to \infty$. In the next section, we will study finite sample behaviour of the tests. We begin

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TABLE 1: Summary of different test statistics for analyzing a set of genetic variants.

Proposed approach		Equivalence[a] with previous methods based on individual-level data	
Regression models	Test statistics	Methods	Test statistics, for example,
Fixed-effect model, Section 3.1		Linear T_1	
$s = \mu w + \varepsilon, \varepsilon \sim N(\mathbf{0}, \mathbf{\Sigma_0})$ (3) $H_0 : \mu = 0$	T_{FE} (5)	let w be $\mathbf{\Sigma_0} w$	Sum-test/burden, Pan (2009) Weighted-sum, Madsen & Browning (2009)
Random-effect model, Section 3.1		Quadratic T_2	
$s = \eta + \varepsilon, \eta \sim N(\mathbf{0}, \tau^2 \boldsymbol{R})$ (6) $H_0 : \tau^2 = 0$	T_{RE} (8)	let $\boldsymbol{R}$ be $\mathbf{\Sigma_0} \boldsymbol{A} \mathbf{\Sigma_0}$	SSU, Pan (2009) SKAT, Wu et al. (2011)
Mixed-effect model, Section 3.2		Hybrid T_1 "+" T_2	
$s = \mu w + \eta + \varepsilon$ (9) $H_0 : \mu = 0, \tau^2 = 0$	T_{ME} (11) T_{12} (12)		T_{skato}, Lee, Wu & Lin (2012) T_{Fisher}, T_{Minp}, Derkach, Lawless & Sun (2013)
Covariate adjustment, Section 3.3			
$s = \mu w + \theta z + \eta + \varepsilon$ (13) $H_0 : \mu = 0, \theta = 0, \tau^2 = 0$	$T_{ME,cov}$ (14)		

[a]The equivalence can be analytical or numerical; see corresponding sessions for details. See Table 1 of Derkach, Lawless & Sun (2014) for a more detailed summary of the previous methods.

with some notations and mild conditions needed for Theorem 1. Let $\boldsymbol{C}$ be a matrix such that $\boldsymbol{C\Sigma_0 C}^T = \boldsymbol{I_J}$, and let $\lambda_{min}(\cdot)$ and $\lambda_{max}(\cdot)$ be the minimum and maximum eigenvalues of a matrix, respectively. For two sequences of real numbers $\{a_{1J}\}$ and $\{a_{2J}\}$, denote $a_{1J} = o(a_{2J})$ if $\lim_{J\to\infty}(a_{1J}/a_{2J}) = 0$.

Condition 1. $c_0^{-1} \le \lambda_{min}(\boldsymbol{\Sigma_0}) \le \lambda_{max}(\boldsymbol{\Sigma_0}) \le c_0$ *for some constant* c_0.

Condition 2. $c^{-1} \le \lambda_{min}(\boldsymbol{R}) \le \lambda_{max}(\boldsymbol{R}) \le c$ *for some constant* c.

Condition 3. $w_{max} = o(\sqrt{\boldsymbol{w'w}})$ *where* $w_{max} = \max_j w_j$.

Theorem 1. *Under the null hypothesis of (10) and assuming Conditions 1–3 hold,*

$$T_{ME} \xrightarrow{d} \chi^2_2 \text{ and } T_{12} \xrightarrow{d} \chi^2_2, \text{ as } J \to \infty.$$

See the Supplementary Material for the proofs of Theorem 1 and all other theorems. When J is small, significance evaluation based on the asymptotic distributions may not be adequate. Theorem 2 provides an approximation for the finite-sample distribution of T_{ME}; the result for T_{12} is similar.

Theorem 2. *Let* λ_j *be the* j*th eigenvalue of* $\boldsymbol{CRC}^T$, $j = 1, \ldots, J$, *then*

$$T_{ME} \overset{D}{=} u_1^2 + \left(2\sum_{j=1}^{J}\lambda_j^2\right)^{-1}\left(\sum_{j=1}^{J}\lambda_j(v_j^2 - 1)\right)^2,$$

where u_1 *and* v_j, $j = 1, \ldots, J$, *are independent* $N(0,1)$ *random variables, and* $\overset{D}{=}$ *denotes equality in distribution.*

We note that the above finite and asymptotic results are with respect to J. The validities of T_{ME} and T_{12} do not require $n \to \infty$ explicitly, as long as $\boldsymbol{s}$ is multivariate normal.

3.5. Power Comparison

In this section, we first establish the asymptotic distributions of T_{ME} and T_{12} (as $J \to \infty$) under alternatives.

Theorem 3. *Under the alternative hypothesis* $H_1 : \mu = \mu_1, \tau^2 = \tau_1^2$,

(a) Assume Conditions 1–3 hold, then

$$T_{ME} \xrightarrow{d} \pi_0^2\chi_1^2\left(\varphi_0^2/\pi_0^2\right) + \pi_1^2\chi_1^2\left((\varphi_1+\varphi_2)^2/\pi_1^2\right), \text{ as } J \to \infty,$$

where $\varphi_0 = \mu_1\sqrt{\boldsymbol{w^T\Sigma_0^{-1}w}}$, $\varphi_1 = 2^{-1/2}\tau_1^2\sqrt{tr(\boldsymbol{\Sigma_0^{-1}R})^2}$,
$\varphi_2 = \mu_1^2\boldsymbol{w^T\Sigma_0^{-1}R\Sigma_0^{-1}w}\Big/\sqrt{2tr(\boldsymbol{\Sigma_0^{-1}R})^2}$,
$\pi_0^2 = 1 + \tau_1^2\boldsymbol{w^T\Sigma_0^{-1}R\Sigma_0^{-1}w}\big/\boldsymbol{w^T\Sigma_0^{-1}w}$, *and*
$\pi_1^2 = 1 + \left(\tau_1^4 tr(\boldsymbol{\Sigma_0^{-1}R})^4 + 2\tau_1^2 tr(\boldsymbol{\Sigma_0^{-1}R})^3\right)\big/tr(\boldsymbol{\Sigma_0^{-1}R})^2$.

(b) Assume Conditions 1–3 hold, then

$$T_{12} \xrightarrow{d} \breve{\pi}_0^2 \chi_1^2 \left(\breve{\varphi}_0^2 / \breve{\pi}_0^2\right) + \breve{\pi}_1^2 \chi_1^2 \left((\breve{\varphi}_1 + \breve{\varphi}_2)^2 / \breve{\pi}_1^2\right), \text{ as } J \to \infty,$$

where $\breve{\varphi}_0 = \mu_1 \boldsymbol{w}^T\boldsymbol{w} / \sqrt{\boldsymbol{w}^T\boldsymbol{\Sigma_0}\boldsymbol{w}}$, $\breve{\varphi}_1 = \tau_1^2 tr(\boldsymbol{R}^2) / \sqrt{2tr(\boldsymbol{\Sigma_0}\boldsymbol{R})^2}$, $\breve{\varphi}_2 = \mu_1^2 \boldsymbol{w}^T\boldsymbol{R}\boldsymbol{w} / \sqrt{2tr(\boldsymbol{\Sigma_0}\boldsymbol{R})^2}$, $\breve{\pi}_0^2 = 1 + \tau_1^2 \boldsymbol{w}^T\boldsymbol{R}\boldsymbol{w} / \boldsymbol{w}^T\boldsymbol{\Sigma_0}\boldsymbol{w}$, *and* $\breve{\pi}_1^2 = 1 + \left(\tau_1^4 tr(\boldsymbol{R}^4) + 2\tau_1^2 tr(\boldsymbol{\Sigma_0}\boldsymbol{R}^3)\right) / tr(\boldsymbol{\Sigma_0}\boldsymbol{R})^2$.

To compare the asymptotic power between T_{ME} and T_{12}, first let us consider the simple case of no random effect, that is, $\tau_1^2 = 0$. In that case, $\pi_0 = \breve{\pi}_0 = \pi_1 = \breve{\pi}_1 = 1$, and we can show that T_{ME} is at least as powerful as T_{12} provided that $\varphi_0^2 / \breve{\varphi}_0^2 = \boldsymbol{w}^T\boldsymbol{\Sigma_0^{-1}}\boldsymbol{w}\boldsymbol{w}^T\boldsymbol{\Sigma_0}\boldsymbol{w} / (\boldsymbol{w}^T\boldsymbol{w})^2 \geqslant 1$. In fact, this inequality holds as long as $\boldsymbol{\Sigma_0}$ is a positive definite symmetric matrix. This suggests that if the true underlying model for $\boldsymbol{s}$ is a fixed-effect model, then T_{ME} is more powerful than T_{12}. Our analytical conclusion here is consistent with that observed by Liu & Lin (2018a) for joint analyses of multiple phenotypes and by Brockwell & Gordon (2011) for meta-analyses.

We next consider a local alternative assuming

$$H_{1J} : \mu_1^2 \boldsymbol{w}^T\boldsymbol{R}\boldsymbol{w} = o\left(\sqrt{2tr(\boldsymbol{\Sigma_0}\boldsymbol{R})^2}\right), \ \tau_1^2 = O(J^{-1/2}).$$

In this case, $\varphi_2 = o(1)$, $\breve{\varphi}_2 = o(1)$, $\pi_0 = 1 + o(1)$, $\breve{\pi}_0 = 1 + o(1)$, $\pi_1 = 1 + o(1)$, and $\breve{\pi}_1 = 1 + o(1)$ based on Conditions 1 and 2. As a result, T_{ME} is at least as powerful as T_{12}, provided that $\varphi_0^2 / \breve{\varphi}_0^2 = \boldsymbol{w}^T\boldsymbol{\Sigma_0^{-1}}\boldsymbol{w}\boldsymbol{w}^T\boldsymbol{\Sigma_0}\boldsymbol{w} / (\boldsymbol{w}^T\boldsymbol{w})^2 \geqslant 1$ and $\varphi_1^2 / \breve{\varphi}_1^2 = tr(\boldsymbol{\Sigma_0^{-1}}\boldsymbol{R})^2 tr(\boldsymbol{\Sigma_0}\boldsymbol{R})^2 / tr^2(\boldsymbol{R}^2) \geqslant 1$. These two inequalities always hold as long as $\boldsymbol{R}$ and $\boldsymbol{\Sigma_0}$ are positive definite symmetric matrices.

4. SIMULATION STUDIES

To compare the finite-sample performance of T_{ME} and T_{12}, with T_{skato}, T_{Minp}, and T_{Fisher}, we conduct extensive simulation studies, examining the effects of different correlation structures and signal sparsities on power.

To obtain the summary statistics, $\boldsymbol{s}$ and $\boldsymbol{\Sigma_0}$, for association studies of rare variants, similar to Derkach, Lawless & Sun (2014) we assume that the underlying individual-level model is $E(y_i|\boldsymbol{G}_i) = \beta_0 + \beta_1 G_{i1} + \cdots + \beta_J G_{iJ}$, where y_i is normally distributed with variance $\sigma^2 = 1$, G_{ij} is Bernoulli with $Pr(G_{ij} = 1) = p_j$ where p_j is approximately twice the MAF of variant j, and $i = 1, \ldots, n$ and $j = 1, \ldots, J$. Given this set-up, $\boldsymbol{s} \sim N(\boldsymbol{\mu}, \boldsymbol{\Sigma_0})$, where $\boldsymbol{\mu} = (np_1(1-p_1)\beta_1, \ldots, np_J(1-p_J)\beta_J)'$, $\boldsymbol{\Sigma}_0 = \{\sigma_{jk}^2\}_{J\times J}$, $\sigma_{jk}^2 = np_j(1-p_j)$ for $j = k$ and $\sigma_{jk}^2 = n(p_{jk} - p_j p_k)$ for $j \neq k$, and $p_{jk} = P(G_{ij} = 1, G_{ik} = 1)$.

We consider $n = 500$ and $J = 10,\ 50,\ 100,\ 500$, and 1,000, and we draw p_j randomly from Unif $(0.005, 0.02)$, $j = 1, \ldots, J$. For $\text{diag}\{\sigma_{jj}^{-1}\}\boldsymbol{\Sigma_0}\ \text{diag}\{\sigma_{jj}^{-1}\}$, we consider an AR(1) pattern with correlation $\tilde{\rho}$, and $\tilde{\rho} = 0.2$, 0.5, and 0.8. For $\boldsymbol{w}$ and $\boldsymbol{A}$ in T_{skato}, T_{Minp}, T_{Fisher}, and T_{12}, we choose the commonly used $w_j = 1/\sqrt{p_j(1-p_j)}$ and $\boldsymbol{A} = \text{diag}\{w_j^2\}$. For $\boldsymbol{w}$ and $\boldsymbol{R}$ in T_{ME}, we choose the same w_j and let $\boldsymbol{R} = \boldsymbol{A}$ for a fair comparison.

4.1. Type I Error

To examine the validity of the proposed tests, T_{ME} and T_{12}, we generate $\boldsymbol{s}$ from $N(\boldsymbol{0}, \boldsymbol{\Sigma_0})$, independently, 10^6 times for each J and $\tilde{\rho}$ combination. The results in Table 2 show that for small J, in combination with stringent α level, the asymptotic P-values are not adequate. In that case, the approximate solution in Theorem 2 should be used.

For the existing methods, T_{skato}, T_{Minp}, and T_{Fisher}, we observe in our simulation studies that T_{skato} is slightly conservative for the α levels considered regardless of the size of J. T_{Minp} is also

TABLE 2: Type I error evaluation.

J	α	$T_{12,asy}$	$T_{me,asy}$	$T_{12,apr}$	$T_{me,apr}$
10	5%	6.1558	5.6556	5.7470	5.2226
	1%	3.1219	2.4281	1.7649	1.2969
	0.1%	1.4531	0.9427	0.2079	0.1316
	0.01%	0.7725	0.4294	0.0196	0.0141
20	5%	5.6845	5.5795	5.4308	5.2198
	1%	2.5849	2.4312	1.6450	1.3667
	0.1%	1.0219	0.9643	0.2212	0.1476
	0.01%	0.4703	0.4550	0.0246	0.0149
50	5%	5.1085	5.1317	5.0333	4.9875
	1%	1.9088	1.7082	1.4498	1.1475
	0.1%	0.5952	0.5002	0.2103	0.1307
	0.01%	0.2148	0.1794	0.0230	0.0132
100	5%	4.9508	5.0064	4.923	4.9664
	1%	1.5279	1.3854	1.2788	1.1030
	0.1%	0.3796	0.3082	0.1846	0.1273
	0.01%	0.1149	0.0831	0.0250	0.0138
500	5%	4.9335	5.0020	4.9307	4.9948
	1%	1.1265	1.0917	1.0720	1.0238
	0.1%	0.1662	0.1547	0.1288	0.1130
	0.01%	0.0332	0.0281	0.0202	0.0139
1,000	5%	4.9841	4.9552	4.9743	4.9436
	1%	1.0475	1.0254	1.0151	0.9864
	0.1%	0.1365	0.1190	0.1183	0.0951
	0.01%	0.0205	0.0162	0.0163	0.0109

Empirical test sizes for $\alpha = 5\%$, 1%, 0.1%, and 0.01%, estimated based on 10^6 replications independently simulated under the null. $T_{.,asy}$ represents the P-value calculation using the asymptotic distributions in Theorem 1, and $T_{.,apr}$ is for the P-value estimation based on Theorem 2 with 10^7 independent u_1 and v_j standard normal variables generated for each of the 10^6 simulated null replicates. Results here are for $\tilde{\rho} = 0.5$; results for other $\tilde{\rho}$ values are provided in the Supplementary Material.

slightly conservative for small J but has correct test size when $J > 50$, and T_{Fisher} has inflated type I error when the correlation among variants is strong (see the Supplementary Material). Our observations here are largely consistent with previous reports (e.g., Larson et al., 2017).

4.2. Power Without Covariates

We consider two different simulation designs to evaluate power. For both designs, P_c, the proportion of causal variants for a given set of J variants, is randomly drawn from Unif$(0.01, 0.1)$ for the case of sparse signals, and $P_c \sim$ Unif $(0.1, 0.5)$ for the case of moderately sparse

signals. Among the causal variants, p_d is the proportion of deleterious variants with $\beta_j > 0$ and $P_d \sim \text{Unif}(0.5, 0.75)$. We note that for each P_c and P_d combination, the locations of the signals ($\beta_j \neq 0$) are randomly drawn from $\{1, 2, \ldots, J\}$, without replacement. This randomness helps us to comprehensively explore the effect of different correlation structures between causal variants, between non-causal variants, as well as between causal and non-causal variants on power.

Design one follows the approach of Derkach, Lawless & Sun (2014). That is, $\boldsymbol{s} \sim N(\boldsymbol{\mu}, \boldsymbol{\Sigma}_0)$ and $|\beta_j| \sim \text{Unif}(0.5, 1.5)$ for both the sparse and moderately sparse cases. Design two assumes that $\boldsymbol{s}$ is drawn from the mixed-effect model (9) with varying magnitudes of τ^2. Specifically, for a given J we first simulate $p_j \sim \text{Unif}(0.005, 0.02), j = 1, \ldots J$, $P_c \sim \text{Unif}(0.1, 0.5)$, and $P_d \sim \text{Unif}(0.5, 0.75)$, and we draw the locations of the causal variants from $\{1, 2, \ldots, J\}$ as in design one. We then specify $\text{sign}(\beta_j)$ accordingly and obtain $\boldsymbol{w}^* = (np_1(1-p_1)\text{sign}(\beta_1), \ldots, np_J(1-p_J)\text{sign}(\beta_J))'$; for a null variant $\text{sign}(\beta_j) = 0$. Finally, we simulate $\boldsymbol{s} \sim N(\beta \boldsymbol{w}^*, \tau^2 \boldsymbol{I}_J + \boldsymbol{\Sigma}_0)$, where $\beta \sim \text{Unif}(0.5, 1.5)$ for both the sparse and moderately sparse cases, and $\tau^2 \sim \text{Unif}(1, 2)$.

For both designs, results of power comparison focus on $J = 100$, $\alpha = 0.05$ and the sparse alternatives; results for the moderately sparse alternatives are characteristically similar (see the Supplementary Material). The empirical power for $\alpha = 0.05$ is estimated from 10^3 simulated replicates, and using the empirical critical values obtained from 10^4 null replicates.

Figure 1a and b shows that when the correlation among variants is relatively strong (e.g., $\tilde{\rho} = 0.5$ in Figure 1a and $\tilde{\rho} = 0.8$ in the Supplementary Material), T_{ME} (based on $\boldsymbol{\Sigma}_0^{-1}\boldsymbol{s}$) has better power than the other methods (based on $\boldsymbol{s}$), including T_{skato}. However, this approach may not be advantageous when there is only weak correlation in conjunction with sparse signal (e.g., $\tilde{\rho} = 0.2$ in Figure 1b) as discussed in Section 3.2. Interestingly, the new T_{12} test (inspired by T_{ME} but based on $\boldsymbol{s}$) has comparable power with T_{skato}, but without the need to search for the "optimal" ρ. Our simulation results here also confirm that the three existing hybrid tests, T_{skato}, T_{Minp}, and T_{Fisher}, have similar performance, where T_{skato} and T_{Minp} perform more similarly with each other than with T_{Fisher}.

Figure 1c and d shows that when $\boldsymbol{s}$ follows a mixed-effect model, the advantage of the proposed T_{ME} is enhanced. In this case, power of T_{ME} is considerably higher than the other tests even when $\tilde{\rho} = 0.2$ (Figure 1d).

4.3. Power with Covariates

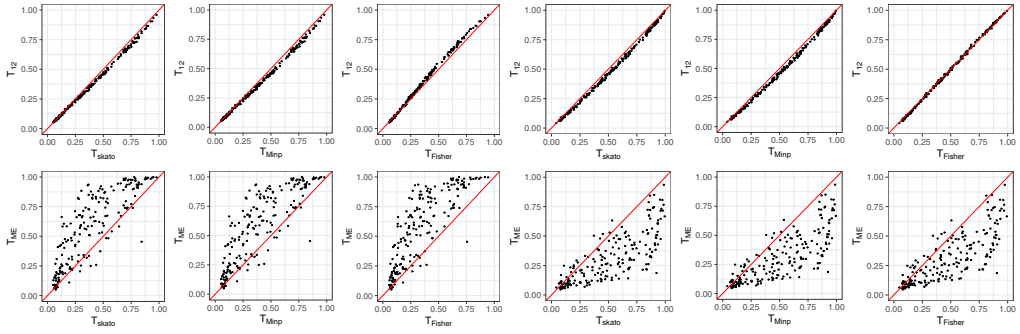
We now briefly study the effect of incorporating variant-specific additional information $\boldsymbol{z} = (z_1, \ldots, z_J)^T$. As discussed before, although one may revise w_j to be proportional to z_j in addition to MAF, it is not immediately clear how to choose an "optimal" weighting function. Thus, we only study the proposed $T_{ME,cov}$, derived directly from the regression model (13), and we consider simulation design two only.

Without loss of generality, we assume z_j to be an indicator variable, for example indicating if the variant is non-synonymous ($z_j = 1$) or synonymous ($z_j = 0$). For causal variants we let $Pr(z_j = 1) = 0.5$, and for non-causal variants $Pr(z_j = 1) = 0$. We consider both the case of informative $\boldsymbol{z}$ ($\theta \neq 0$ in model (13) and $\theta \sim \text{Unif}(1, 4)$) and the case of uninformative $\boldsymbol{z}$ ($\theta = 0$). Because of the additional information available from $\boldsymbol{z}$, we draw β from $\text{Unif}(0.1, 1)$ and choose $\tau^2 = 0$. We also assumed $P_c = 0.1$ and $P_d \sim \text{Unif}(0.5, 1)$ for this set of simulations.

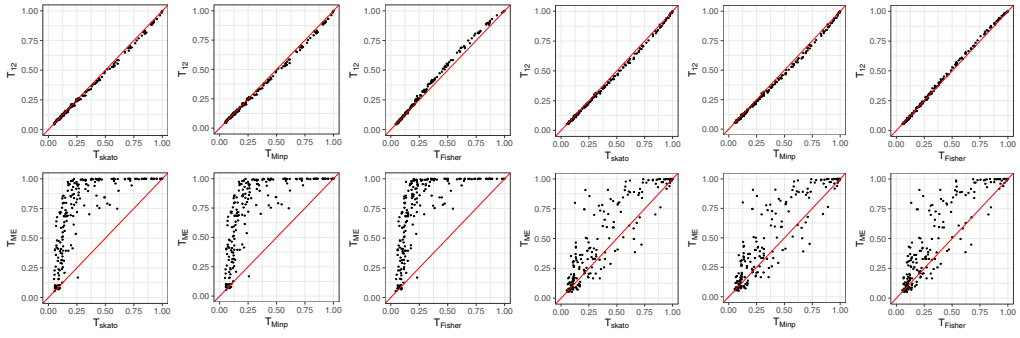
Figure 2 shows that, as expected, there can be substantial power gain when incorporating informative covariate information (the left plot), at the cost of slightly reduced power when $\boldsymbol{z}$ is uninformative (the right plot).

5. APPLICATIONS

In this section, we examine eight test statistics including $T_{ME,cov}$, T_{ME}, and T_{12}, and the existing methods, T_{skato}, T_{Minp}, T_{Fisher}, as well as T_1 and T_2 for completeness through two



(a) Design ONE, $\tilde{\rho} = 0.5$ (b) Design ONE, $\tilde{\rho} = 0.2$



(c) Design TWO, $\tilde{\rho} = 0.5$ (d) Design TWO, $\tilde{\rho} = 0.2$

FIGURE 1: Power comparison under sparse alternatives based on designs ONE and TWO, comparing T_{12} and T_{ME} with T_{Fisher}, T_{Minp}, and T_{skato}. Empirical power of 200 alternative models is shown. For each model, $J = 100$ and the proportion of the causal variants varies from 1% to 10% randomly. See the main text for additional simulation details.

data applications. In the implementation of $T_{ME,cov}$, we use variants, being non-synonymous or synonymous, annotated using the UCSC genome browser at https://genome.ucsc.edu/, as the variant-specific information.

The first application highlights the advantage of the proposed T_{ME} in the presence of high or moderately high correlation between variants, and it also demonstrates that the method is not limited to analyses of rare variants. The second application revisits the genetic analysis workshop 17 (GAW17) rare variants data previously studied by Derkach, Lawless & Sun (2014). This application reveals the benefit of incorporating covariate information using $T_{ME,cov}$. For both applications, we used the method of Rothman (2012) to achieve a positive definite estimate for the covariance matrix $\boldsymbol{\Sigma}_0$.

5.1. Cystic Fibrosis (CF) Data—Common Variants

Cystic fibrosis is a life-limiting genetic condition for which lung function is a primary co-morbidity of interest. To *indirectly* study gene-environment interactions, Soave et al. (2015) proposed a joint location-scale (JLS) test and applied it to lung function measures in CF individuals; $n = 1,409$ from a Canadian sample and $n = 1,232$ from a French sample. They discovered and replicated the significance of the SLC9A3 complex set (35 common variants from four genes) based on the JLS test. However, the association evidence appears to come from the

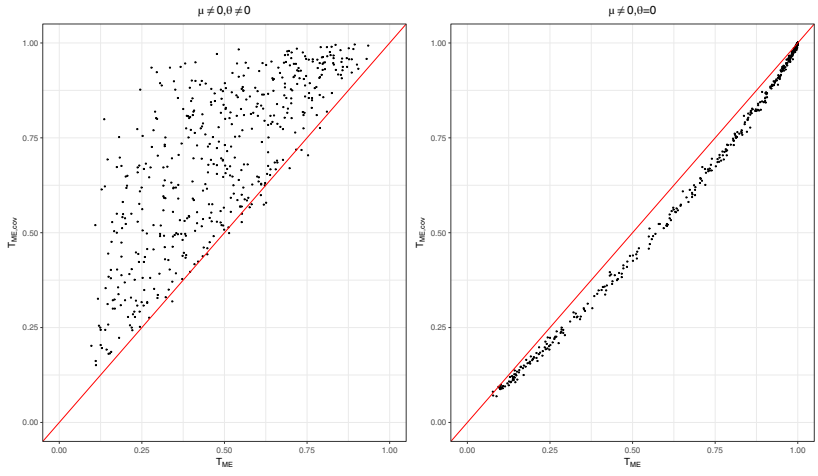


FIGURE 2: Power comparison under sparse alternatives based on design TWO, comparing $T_{ME,cov}$ with T_{ME}. The left plot is for the case of an informative covariate ($\theta \neq 0$), and the right plot is for an uninformative covariate ($\theta = 0$). Empirical power of 500 alternative models is shown. For each model, $J = 100$ and the proportion of the causal variants is 10%, and $\tilde{\rho} = 0.5$. See the main text for additional simulation details.

TABLE 3: Empirical P-values of the tests in the CF data application.

		Empirical P-values							
Gene	J	T_1	T_2	T_{skato}	T_{Minp}	T_{Fisher}	T_{12}	T_{ME}	$T_{ME,cov}$
SLC9A3	7	**0.0443**	0.1198	0.0790	0.0783	0.0541	0.0718	0.0886	0.0860
EZR	10	0.2192	0.6856	0.3241	0.3209	0.3897	0.2390	0.3473	0.3474
SLC9A3R2	10	0.8040	0.5951	0.6965	0.6984	0.7090	0.9037	0.9683	0.8791
SLC9A3R1	8	0.0999	0.1103	0.1471	0.1656	0.0846	0.1042	**0.0243**	**0.0243**
4-Gene jointly	35	0.8372	0.3079	0.4749	0.4738	0.5671	0.9261	0.1710	0.1709

scale (interaction) component. For the traditional location (mean) test based on T_2, the SLC9A3 complex set was only significant in the Canadian sample but not replicated in the French sample. Here we examine the performance of the eight tests applied to the French sample. For a fair comparison, we use $\boldsymbol{w} = \mathbf{1}, \boldsymbol{A} = \boldsymbol{I}$, and $\boldsymbol{R} = \boldsymbol{I}$ for all corresponding tests, and we obtain empirical P-values for all tests based on 10^4 permutation replicates.

Results in Table 3 show that only some of the genes appear to be truly associated with CF lung function. For SLC9A3, all tests have suggestive evidence with T_1 having P-value < 0.05. For SLC9A3R1, benefiting from the correlation structure (as in the Supplementary Material) the proposed T_{ME} and $T_{ME,cov}$, which use $\boldsymbol{\Sigma}_0^{-1}\boldsymbol{s}$ instead of $\boldsymbol{s}$, are significant. When jointly analyzing all four genes in the SLC9A3 complex set, none of the tests is statistically significant, but T_{ME} has the smallest P-value. A larger sample is needed to make a definitive conclusion of true association. The covariate information (non-synonymous vs. synonymous) appears not to be informative here, but the performance of $T_{ME,cov}$ is similar to that of T_{ME}.

TABLE 4: Empirical power of the tests in the GAW17 data application.

Gene	J_C	J_N	Empirical power							
			T_1	T_2	T_{skato}	T_{Minp}	T_{Fisher}	T_{12}	T_{ME}	$T_{ME,cov}$
7 genes for which the maximum power is 10% or more										
SIRT1	4	7	0.440	0.385	0.455	0.430	0.495	**0.500**	0.285	0.285
BCHE	6	9	0.290	0.390	0.405	0.390	0.435	0.445	**0.460**	0.410
PDGFD	3	5	0.295	0.385	0.385	0.380	0.425	**0.450**	0.310	0.260
SREBF1	4	5	**0.495**	0.250	0.440	0.440	0.445	0.400	0.405	0.355
RARB	1	5	0.060	0.135	0.095	0.110	0.085	0.090	0.100	**0.215**
PLAT	4	7	0.130	0.125	0.105	0.100	0.135	0.120	0.130	**0.165**
VLDLR	4	3	0.110	0.085	0.100	0.095	0.115	0.115	0.110	**0.160**
7-Gene jointly	26	41	0.935	0.765	0.940	0.935	**0.945**	0.940	0.765	0.765
4 genes for which the maximum power is 10% or less										
VNN3	2	2	0.035	0.040	0.035	0.035	0.040	0.040	0.040	0.035
INSIG1	3	1	0.050	0.030	0.030	0.030	0.035	0.035	0.025	0.015
LPL	1	3	0.030	0.065	0.035	0.035	0.040	0.035	0.035	0.050
VWF	1	3	0.025	0.010	0.010	0.010	0.015	0.010	0.040	0.045
4-Gene jointly	7	9	0.075	0.010	0.060	0.060	0.055	0.045	0.055	0.045
1 gene for which there is no rare causal variants, used as a negative control										
VNN1	0	3	0.015	0.045	0.045	0.045	0.035	0.040	0.040	0.045

5.2. The Genetic Analysis Workshop 17 (GAW17) Data—Rare Variants

Here we apply the method to the GAW17 data provided by the 1000 Genomes Project (1000 Genomes Project Consortium et al., 2010; Almasy et al., 2011), focusing on the simulated quantitative trait Q2. The phenotype Q2 is influenced by 72 variants in 13 genes but not by environmental factors, and the genotypes of these variants are obtained from a "mini-exome" next-generation sequencing experiment (1000 Genomes Project Consortium et al., 2010). Available to us are 200 replicates, simulated based on a true phenotype–genotype association model determined by the GAW17 study group (Almasy et al., 2011) but blinded to this analysis. We consider $n = 321$ unrelated Asian samples and use only variants with MAF less than 0.05. The description of the variants is provided in Table 4. Among the 13 genes, GCKR is not analyzed, since only one variant remained after variant screening. VNN1 does not have any causal rare variants but is kept for negative control.

For each of the 200 alternative replicates, we calculate the empirical P-values (based on 10^4 permutation replicates) for the eight test statistics. For each test, the power for $\alpha = 0.05$ is estimated as the proportion of the 200 replicates for which the empirical P-values ≤ 0.05. We separate the 11 genes into three categories based on power as in Derkach, Lawless & Sun (2014) (which examined T_1 and T_2) and Derkach, Lawless & Sun (2013) (which examined T_1, T_2, T_{Minp}, and T_{Fisher}), and we also jointly analyze all genes within each category.

In this application, because the correlation is weak among variants (see the Supplementary Material), we anticipate that methods relying on $\boldsymbol{s}$ will have better power than those based

on $\boldsymbol{\Sigma}_0^{-1}\boldsymbol{s}$. Indeed, results in Table 4 show that T_{12} has better performance than T_{ME}. However, this application clearly demonstrates the potential of incorporating informative covariates. For example, the power of analyzing RARB, PLAT, and VLDLR is higher using $T_{ME,cov}$, at the cost of slightly reduced performance for other genes when the included covariate is presumably not (detectably) informative. Interestingly, T_{Fisher} has comparable performance to T_{12}, and both outperform T_{skato} in almost all cases. Although the individual T_1 and T_2 tests may have the highest power for certain genes, the robustness of the hybrid tests is evident based on the overall performance exhibited in Table 4.

6. DISCUSSION

In this article, we considered a summary statistics-based regression framework to jointly analyze a set of J variants. As delineated in Table 1, the proposed approach is flexible and adaptive. The score test derived from the fixed-effect model, T_{FE}, unifies the linear class of tests (also known as the burden tests), T_1, while T_{RE} from the random-effect model connects the quadratic class or variance component tests, T_2. Furthermore, the score test derived from the random-effect model offers a new hybrid test, T_{ME}, that naturally aggregates information from T_{FE} and T_{RE}.

It is worth emphasizing three notable differences between the proposed T_{ME} and the well-known T_{skato} (Lee, Wu & Lin, 2012). First, the proposed framework aggregates evidence across J variants based on $\boldsymbol{\Sigma}_0^{-1}\boldsymbol{s}$, a precision matrix-based transformation of the score vector, that can increase power for detecting sparse alternatives (Fan, Jin & Yao, 2013; Cai, Liu & Xia, 2014). Second, unlike T_{skato}, T_{ME} naturally aggregates the information from the linear and quadratic types of tests, based on a regression model *without* searching for the "optimal" weight. Lastly, when additional variant-specific information is available, it is straightforward to derive a $T_{ME,cov}$ that accounts for covariate effects. We have demonstrated these features of the proposed method both analytically and empirically.

To exploit the assumption of signal sparsity, various supremum−type tests have been proposed, including the generalized higher criticism (Wu et al., 2014; Barnett, Mukherjee & Lin, 2017) for sparse signals, and most recently the generalized Berk−Jones statistic (Sun & Lin, 2019) for moderately sparse signals. These methods, tailored for common variants, are not easy to adjust for additional variant-specific information.

The proposed set-based testing framework is a general one, and it can be used for other settings such as pleiotropy studies of multiple phenotypes, where the analytical unit is each of the phenotypes. In that context, Liu & Lin (2018b) also proposed a summary statistics—based linear mixed—effect regression model, but they focused on the special case of $\boldsymbol{w} = \mathbf{1}$ and $\boldsymbol{R} = \boldsymbol{I}$. In addition, Liu & Lin (2018b) derived two score test statistics, respectively, for testing $\mu = 0$ and $\tau^2 = 0$ *separately*, then considered different ways to combine the evidence including the SKAT-O type of statistics. In contrast, we derive T_{ME} from testing $\mu = 0$ and $\tau^2 = 0$ *jointly*, and the weighting factors are inherently justified based on the regression model. We also study the asymptotic properties of the proposed tests under the alternatives as well as empirical property under sparse alternatives.

The proposed method can also be used for the study of PRS (Purcell et al., 2009), and the connection between PRS and T_1 has been noted by Pan, Chen & Wei (2015). In principle, T_{ME} can overcome the poor statistical efficiency of T_1, but accurate estimation of large precision matrices can be challenging and requires special considerations (Fan, Liao & Liu, 2016).

The link between T_1 and PrediXcan (Gamazon et al., 2015) and TWAS (Gusev et al., 2016) for association and tissue-specific gene-expression data integration has also been noted (Xu et al., 2017). The performance of T_{ME} in this setting and comparison with other concurrently developed newer methods are of our future research interest.

Fixed-, random-, and mixed-effect models for summary statistics have been studied for meta-analyses of GWAS (Han & Eskin, 2011). In that context, a likelihood ratio test was

implemented for a mixed-effect model, and the resulting test is also known as the new RE meta-analysis. The original test of Han & Eskin (2011) was designed for meta-analyses of independent studies, and a modified procedure has since been developed by Lee, Eskin & Han (2017) to account for correlations between studies but without adjusting for covariate effects. Comparisons between the two approaches for meta-analyses and other studies warrant future investigations.

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