Supplementary material for

“Fast and accurate genome-wide association test of multiple continuous traits”

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1 CCA as a score test under MLM

When there are no covariates, CCA is typically applied to test the multi-trait association. Here we show that CCA is equivalent to a score statistic under the MLM. Without loss of generality, assume the genotype and individual trait have been centered. For sample $i = 1, \ldots, n$, consider

$$y_{ki} = g_i \beta_k + \epsilon_{ki}, \quad k = 1, \ldots, m,$$

where the error vector $(\epsilon_{1i}, \ldots, \epsilon_{mi})^T$ follows a zero-mean multivariate normal distribution with covariance matrix $\Sigma$, where variance $\text{Var}(\epsilon_{ki}) = \sigma^2_k$ and covariance $\text{Cov}(\epsilon_{ki}, \epsilon_{ji}) = \sigma_{kj}$. Denote $Y_i = (y_{1i}, \ldots, y_{mi})^T$, $Y = (Y_1, \ldots, Y_n)$, $G = (g_1, \ldots, g_n)^T$, and $\beta = (\beta_1, \ldots, \beta_m)^T$. 
The log likelihood is proportional to

$$\ell = -n \log |\Sigma| - \sum_{i=1}^{n} (Y_i - g_i \beta)^T \Sigma^{-1} (Y_i - g_i \beta).$$

Hence we can easily show that the score vector for testing $H_0: \beta = 0$ is

$$U = \sum_{i=1}^{n} g_i \Sigma^{-1} Y_i = \Sigma^{-1} (YG) = (G^T \otimes \Sigma^{-1}) vec(Y),$$

where $\otimes$ means the matrix Kronecker product, and $vec()$ is the vector operator which stacks the columns of a matrix into a column vector. Note $Cov[vec(Y)] = I \otimes \Sigma$. Hence

$$Cov(U) = (G^T G) \otimes \Sigma^{-1} = n S_{11} \Sigma^{-1},$$

where $S_{11} = G^T G/n$ is the sample variance of genotype. Denote $S_{21} = YG/n$, which is the sample covariance vector of multi-traits and genotype. The multi-trait association can be based on the following chi-square statistic, $U^T \hat{Cov}(U)^{-1} U$, which is

$$Q = n \frac{S_{21}^T S_{22}^{-1} S_{21}}{S_{11}},$$

where we have plugin the estimated null covariance matrix $\hat{\Sigma}$ using the sample covariance matrix $S_{22}$ of multi-traits. The CCA test statistic used in Ferreira and Purcell (2009) is then

$$\frac{Q/n}{1 - Q/n} \frac{n - m - 1}{m}.$$

Therefore the proposed MLM based Wald test can be treated as a natural and flexible generalization of the CCA: (1) it can accommodate any covariates; (2) it is based on the more powerful Wald test instead of the Score test for association tests of quantitative
traits; and (3) it has an exact F-distribution for the multivariate normally distributed multiple continuous traits, and hence has very accurate control of type I errors.

It is not hard to verify that previous derivations still hold when we replace \( Y \) and \( G \) by their residuals regressing on a common set of \( p \) covariates. Therefore operationally we can apply the popular PLINK tool (Purcell et al., 2007) to test multi-trait association as follows. We first obtain the residuals of multivariate traits and genotypes adjusting for all covariates. We then input the residuals into the CCA test approach (Ferreira and Purcell, 2009) implemented in PLINK. Technically we need to adjust the PLINK output p-value \( T \) using a F-distribution with different DFs as 
\[
1 - F_{m,n-m-1-p}(F_{m,n-m-1}^{-1} - T)\frac{n-m-1-p}{n-m-1},
\]
where \( F_{d_1,d_2}(\cdot) \) is the distribution function of F-distribution with \( (d_1, d_2) \) DFs. Note that when the set of covariates are independent of the genotypes (e.g., age and gender), we can directly use the genotypes instead of the genotype residuals, which can further save some computation time.

## 2 Multivariate trait association detection using the 1-DF Wald test

Consider the linear combination \( U = a^T \hat{\beta}_1 \), which follows a normal distribution, \( U \sim N(a^T \gamma, (G_e^T G_e)^{-1} a^T \Sigma a) \), where \( \gamma \) is the true value of \( \beta_1 \). Assuming a common genotype effect across the multivariate traits, we have \( \gamma = \eta 1_m \). The effect size of \( U \) is then proportional to

\[
\frac{\eta(a^T 1_m)}{\sqrt{(G_e^T G_e)^{-1} a^T \Sigma a}} = \eta \sqrt{G_e^T G_e} b^T \Sigma^{-1/2} 1_m, \quad b = \frac{\Sigma^{1/2} a}{\sqrt{a^T \Sigma a}}.
\]
Taking $b \propto \Sigma^{-1/2} \mathbf{1}_m$ will maximize the effect size (note $b^T b = 1$). Therefore we use the following statistic

$$T = \frac{\mathbf{1}_m^T \hat{\Sigma}^{-1} \hat{\beta}_1}{(\mathbf{1}_m^T \hat{\Sigma}^{-1} \mathbf{1}_m)^{1/2}}.$$  

With a common scaled genotype effect across the multivariate traits, we have $\gamma = \eta S$, where $S = (s_1, \ldots, s_m)^T$ with $s_k = \sqrt{\Sigma_{kk}}$, $k = 1, \ldots, m$. Similarly we can derive the following test statistic

$$T' = \frac{S^T \hat{\Sigma}^{-1} \hat{\beta}_1}{(S^T \hat{\Sigma}^{-1} S)^{1/2}}.$$  

### 3 Chi-square and F-distribution based p-value calculation

The chi-square statistic $\frac{n-p-1}{n} Q$ is commonly used in practice and referred to a $m$-DF chi-square distribution to compute multi-trait association test p-values, which can lead to significantly inflated type I errors at stringent genome-wide significance levels. Figure 1 shows the ratio of actual significance level of Wald test p-values computed using the chi-square distribution and F-distribution respectively. We can see that the type I error based on the chi-square distribution is inflated: more so for larger number of traits, smaller significance level, and smaller sample size. For example, when testing $m = 8$ traits with $p = 2$ covariates and $n = 500$ samples, under genome-wide significance level $5 \times 10^{-8}$, the actual significance level of chi-square distribution p-value is $3.42 \times 5 \times 10^{-8} = 1.7 \times 10^{-7}$. Using the chi-square distribution to compute p-values will lead to very small inflation only when the sample size is large, such as in the meta-analysis of multiple GWAS studies. Figure 2 shows the minimum sample size required to have the type I error inflation $\leq 1.1$ at significance level $\alpha = 5 \times 10^{-8}$ as a function of number of traits $m$, when using the chi-square distribution instead of F-distribution to compute p-values. The minimum sample
size almost increases linearly with number of traits \( m \). For typical GWAS with small to medium sample sizes, we recommend using the appropriate F-distribution to compute significance p-values to reduce false positive findings.

4 Joint analysis of glycemic traits in ARIC GWAS

Table 1 lists the 62 genome-wide significant SNPs that were identified in the ARIC joint association test of fasting glucose (FG), fasting insulin (FI) and 2 hour fasting glucose levels (2hFG), and were also significant in the MAGIC consortium meta-analyses of these three traits (Dupuis et al., 2010; Saxena et al., 2010). In Table 1, we listed the ARIC joint test p-values (the proposed MLM Wald test and the GEE chi-square test), and the corresponding MAGIC consortium meta-analyses p-values.
Table 2 lists the 79 novel SNPs that were identified in the ARIC joint association test of FG, IS and 2hFG, but have not been reported as significantly associated with diabetes related fasting glucose and insulin levels before. Among them, one SNP rs4665987 is located on chromosome 2:27755825 and another 78 SNPs are clustered on chromosome 15:62132921 to 15:62396389. Majority of them have large meta-analysis p-values for FG and FI, and relatively small p-values (around $10^{-5}$) for the 2hFG. Interestingly 6 of them (rs4502156, rs7163757, rs8037894, rs6494307, rs7167878, rs7172432) were genome-wide significant in the MAGIC meta analysis of fasting proinsulin level (FP, Strawbridge et al., 2011), with meta-analysis p-values ranging from $3.8 \times 10^{-11}$ to $8.7 \times 10^{-11}$. We have highlighted these 6 SNPs in yellow at Table 2. We also provided the corresponding MAGIC meta-analysis p-values for FG, FI, 2hFG, and FP.
Table 1: Genome-wide significant SNPs that were identified in the ARIC joint association test of FG (fasting glucose), FI (fasting insulin) and 2hFG (2 hour fasting glucose), and were also significant in the MAGIC consortium meta-analyses.

| SNP       | Chr | bp            | ARIC joint test Pval | MAGIC meta-analysis Pval |
|-----------|-----|---------------|----------------------|--------------------------|
|           |     |               | Wald GEE             | FG FI 2hFG               |
| rs1260326 | 2   | 27730940      | 1.0E-09 2.7E-09      | 4.3E-13 1.2E-04 1.5E-06  |
| rs780094  | 2   | 27741237      | 1.4E-10 3.1E-10      | 2.5E-12 9.8E-05 1.5E-06  |
| rs780093  | 2   | 27742603      | 1.5E-10 3.2E-10      | 2.9E-13 2.0E-04 1.7E-06  |
| rs13431652| 2   | 169753415     | 3.8E-11 3.5E-11      | 2.9E-63 1.4E-01 7.8E-01  |
| rs1402837 | 2   | 169757354     | 1.8E-08 1.9E-08      | 7.4E-40 1.2E-01 7.7E-01  |
| rs573225  | 2   | 169757541     | 1.2E-11 1.4E-11      | 6.2E-71 1.4E-01 6.0E-01  |
| rs560887  | 2   | 169763148     | 3.0E-13 3.8E-13      | 4.6E-75 6.2E-02 4.1E-01  |
| rs563694  | 2   | 169774071     | 1.6E-13 2.5E-13      | 1.2E-71 1.2E-01 1.5E-01  |
| rs537183  | 2   | 169774646     | 1.5E-13 2.4E-13      | 9.0E-73 1.1E-01 1.6E-01  |
| rs502570  | 2   | 169774959     | 1.5E-13 2.4E-13      | 9.2E-73 1.1E-01 1.6E-01  |
| rs475612  | 2   | 169776746     | 6.7E-13 7.7E-13      | 1.0E-65 8.0E-02 1.9E-01  |
| rs557462  | 2   | 169777595     | 1.5E-13 2.3E-13      | 3.4E-72 1.1E-01 1.6E-01  |
| rs478333  | 2   | 169779156     | 2.6E-08 2.7E-08      | 3.2E-36 8.2E-02 3.9E-01  |
| rs496550  | 2   | 169779712     | 2.6E-08 2.7E-08      | 1.3E-36 8.7E-02 4.3E-01  |
| rs473351  | 2   | 169779896     | 1.1E-09 1.8E-09      | 5.7E-43 1.1E-01 1.8E-01  |
| rs575671  | 2   | 169780818     | 1.1E-09 1.8E-09      | 5.5E-43 1.1E-01 2.0E-01  |
| rs519887  | 2   | 169780885     | 2.5E-08 2.6E-08      | 2.6E-36 7.3E-02 5.2E-01  |
| rs486981  | 2   | 169782149     | 1.1E-13 2.2E-13      | 2.5E-67 1.1E-01 2.9E-01  |
| rs484066  | 2   | 169782481     | 7.7E-12 9.7E-12      | 1.4E-59 6.9E-02 5.1E-01  |
| rs569805  | 2   | 169782850     | 1.1E-13 2.2E-13      | 2.6E-67 1.1E-01 3.0E-01  |
| rs579060  | 2   | 169783039     | 1.1E-13 2.2E-13      | 2.5E-67 1.2E-01 3.0E-01  |
| rs17540054| 2   | 169784493     | 7.3E-08 4.2E-08      | 8.7E-38 1.0E-01 7.2E-01  |
| rs508560  | 2   | 169784955     | 1.1E-13 2.2E-13      | 2.2E-67 1.2E-01 3.0E-01  |
| rs503931  | 2   | 169785449     | 2.4E-08 2.6E-08      | 5.5E-36 7.7E-02 5.1E-01  |
| rs551754  | 2   | 169787686     | 2.6E-08 2.7E-08      | 2.4E-36 8.4E-02 5.0E-01  |
| rs497692  | 2   | 169789016     | 3.2E-08 3.3E-08      | 1.9E-35 7.4E-02 5.0E-01  |
| rs494874  | 2   | 169789306     | 2.2E-13 3.6E-13      | 3.3E-67 1.3E-01 3.4E-01  |
| rs552976  | 2   | 169791438     | 5.4E-13 7.6E-13      | 7.1E-66 9.6E-02 3.0E-01  |
| rs567074  | 2   | 169794431     | 6.1E-09 6.4E-09      | 2.3E-42 1.0E-01 6.3E-01  |
| rs2544367 | 2   | 169796288     | 3.5E-08 3.6E-08      | 2.1E-37 7.3E-02 6.4E-01  |
| rs2658805 | 2   | 169797060     | 3.5E-08 3.6E-08      | 3.9E-37 7.7E-02 6.4E-01  |
| rs1581397 | 2   | 169797652     | 3.4E-08 3.5E-08      | 1.5E-37 6.7E-02 6.4E-01  |
| rs2685814 | 2   | 169798619     | 3.2E-08 3.4E-08      | 1.3E-37 7.3E-02 6.2E-01  |
| rs853789  | 2   | 169801488     | 1.2E-14 2.5E-14      | 1.9E-67 9.8E-02 2.9E-01  |
| rs860510  | 2   | 169801628     | 3.1E-08 3.2E-08      | 5.2E-38 6.0E-02 6.6E-01  |
| rs853788  | 2   | 169801905     | 3.1E-08 3.2E-08      | 1.6E-38 6.3E-02 6.6E-01  |
| rs853787  | 2   | 169802252     | 1.2E-14 2.5E-14      | 3.7E-73 9.9E-02 3.1E-01  |
| rs853786  | 2   | 169802310     | 3.1E-08 3.2E-08      | 2.3E-38 6.0E-02 6.7E-01  |
| rs862662  | 2   | 169802329     | 2.0E-09 2.3E-09      | 6.7E-44 6.3E-02 6.0E-01  |
Table 2: 79 novel SNPs identified in the ARIC joint association test of FG, FI and 2hFG. The corresponding MAGIC consortium meta-analyses p-values for the three traits together with the FP (fasting proinsulin) are also listed.

| SNP       | Chr | bp       | ARIC joint test Pval | MAGIC meta-analysis Pval |
|-----------|-----|----------|----------------------|--------------------------|
|           |     |          | Wald                 | GEE                      |
| rs4665987 | 2   | 27755825 | 4.9E-08              | 1.0E-07                  |
| rs17271144| 15  | 62132921 | 2.5E-08              | 1.7E-08                  |
| rs3743297 | 15  | 62149784 | 1.5E-08              | 9.1E-09                  |
| rs12908081| 15  | 62162264 | 1.6E-08              | 1.0E-08                  |
| rs1981916 | 15  | 62171479 | 9.1E-09              | 5.4E-09                  |
| rs2414753 | 15  | 62172429 | 9.1E-09              | 5.4E-09                  |
| rs2414753 | 15  | 62200974 | 1.1E-08              | 5.6E-09                  |
| rs963024  | 15  | 62211450 | 2.9E-08              | 1.6E-08                  |
| rs4775453 | 15  | 62217391 | 1.5E-08              | 8.8E-09                  |
| rs4774427 | 15  | 62217444 | 1.2E-08              | 6.0E-09                  |
| rs7172967 | 15  | 62218568 | 1.2E-08              | 6.0E-09                  |
| rs12439934| 15  | 62224613 | 1.9E-08              | 1.4E-08                  |
| rs11071642| 15  | 62229356 | 1.3E-08              | 6.6E-09                  |
| rs2042608 | 15  | 62232380 | 2.7E-09              | 2.0E-09                  |
| rs8033816 | 15 | 62233167 | 1.9E-08 | 1.5E-08 | 7.4E-02 | 7.9E-01 | 1.3E-05 | 4.1E-04 |
| rs7170293 | 15 | 62236373 | 5.0E-09 | 3.1E-09 | 4.6E-02 | 9.9E-01 | 2.2E-05 | 4.3E-04 |
| rs7177173 | 15 | 62236804 | 6.0E-09 | 3.2E-09 | 9.2E-02 | 9.8E-01 | 1.3E-05 | 3.1E-04 |
| rs1425270 | 15 | 62237710 | 3.9E-09 | 2.5E-09 | 2.0E-02 | 9.1E-01 | 1.5E-05 | 4.2E-04 |
| rs7166891 | 15 | 62239304 | 5.5E-09 | 3.3E-09 | 5.1E-02 | 9.9E-01 | 2.4E-05 | 4.4E-04 |
| rs7172145 | 15 | 62239697 | 5.8E-09 | 3.5E-09 | 5.0E-02 | 1.0E+00 | 2.5E-05 | 4.8E-04 |

| rs4587915 | 15 | 62241962 | 3.9E-09 | 2.5E-09 | 2.0E-02 | 9.1E-01 | 1.5E-05 | 4.2E-04 |
| rs7166891 | 15 | 62239304 | 5.5E-09 | 3.3E-09 | 5.1E-02 | 9.9E-01 | 2.4E-05 | 4.4E-04 |
| rs7177173 | 15 | 62236804 | 6.0E-09 | 3.2E-09 | 9.2E-02 | 9.8E-01 | 1.3E-05 | 3.1E-04 |
| rs8026008 | 15 | 62377805 | 4.6E-09 | 2.0E-09 | 1.2E-01 | 7.7E-01 | 8.4E-06 | 7.4E-04 |
5 Multivariate trait association test with different covariates

For trait $y_k$, denote $x_k$ as its covariate vector (including the intercept) of length $p_k$, $k = 1, \ldots, m$. Here $x_k$ are different across traits. Assume the multivariate regression model, 

$$y_k = x_k^T \beta_{0k} + G \beta_{1k} + \epsilon_k, \quad k = 1, \ldots, m,$$

where $\beta_{0k}$ is of length $p_k$, and $\epsilon = (\epsilon_1, \ldots, \epsilon_m)^T$ follows a zero-mean multivariate normal distribution with covariance $\Sigma$, $\epsilon \sim N(0, \Sigma)$. This model is also known as seemingly unrelated regression (SUR) model or multiple-design multivariate (MDM) model (see, e.g., Timm, 2002, chapter 5).

Given observations of $n$ individuals, denote the $n \times m$ response matrix as $Y$, the $m \times p$ block matrix as $F_i = \text{diag}(F_{1i}^T, \ldots, F_{mi}^T) = \bigoplus_{k=1}^m F_{ik}^T$ for the $i$-th individual, where $p = \sum_{k=1}^m (p_k + 1)$ and $F_{ik} = (x_{ik}^T, G_i)^T$ is a column vector of length $p_k + 1$, and the $n \times m$
error matrix as \( \mathbf{B} = (\mathbf{e}_1, \ldots, \mathbf{e}_n)^T \). Denote the \((nm) \times p\) matrix \( \mathbf{A} = (\mathbf{F}_1^T, \ldots, \mathbf{F}_n^T)^T \), and \( \mathbf{\beta} = (\beta_{01}, \beta_{11}, \ldots, \beta_{0m}, \beta_{1m})^T \) of length \( p \). Then the SUR or MDM model can be written in matrix notation as \( \text{vec}(\mathbf{Y}^T) = \mathbf{A}\mathbf{\beta} + \text{vec}(\mathbf{B}^T) \). The MLEs can be very efficiently solved based on iteration of \( \hat{\mathbf{\beta}} = [\mathbf{A}^T (\mathbf{I}_n \otimes \Sigma^{-1}) \mathbf{A}]^{-1} [\mathbf{A}^T (\mathbf{I}_n \otimes \Sigma^{-1}) \text{vec}(\mathbf{Y}^T)] \) and updating \( \hat{\Sigma} \) as the sample covariance of residuals (see, e.g., Timm, 2002, chapter 5). Note that \( \text{Cov}(\hat{\mathbf{\beta}}) = [\mathbf{A}^T (\mathbf{I}_n \otimes \Sigma^{-1}) \mathbf{A}]^{-1} \). When computing the Wald statistics, we plug in the estimated \( \hat{\Sigma} \) and account for its estimation uncertainty by using an approximate F-distribution to compute p-values (see, e.g., Timm, 2002, p. 313). Here \( \mathbf{A}^T (\mathbf{I}_n \otimes \Sigma^{-1}) \mathbf{A} = \sum_{i=1}^{n} \mathbf{F}_i^T \Sigma^{-1} \mathbf{F}_i \), \( \mathbf{A}^T (\mathbf{I}_n \otimes \Sigma^{-1}) \text{vec}(\mathbf{Y}^T) = \sum_{i=1}^{n} \mathbf{F}_i^T \Sigma^{-1} \mathbf{Y}_i \).

5.1 Simulation study

We consider a common Bernoulli covariate \( Z \) with probability of 0.5 (population indicator), and separately simulate a standard normal covariate \( X_k \) for each trait \( Y_k \). The SNP genotype score \( G \) is simulated from a Binomial distribution, Binom(2, \( f_0 \)), where the minor allele frequency (MAF) \( f_0 = p_0 + p_1 Z \).

We conducted simulations for testing \( m = 2, 4, 8 \) related traits of 1,000 unrelated individuals respectively. Each time we simulate the \( m \) traits from a multivariate normal distribution with a compound symmetry correlation matrix with correlation \( \rho \). The first trait has a variance of 2 and all the other traits have unit variance. We set \( E(Y_i) = 1 + 0.5X_i + 0.5Z + \gamma_i G \) for \( i = 1, \ldots, m-1 \), and \( E(Y_k) = 1 + X_k + Z + \gamma_k G \) for \( k = 2, \ldots, m \).

We used 10 million experiments to evaluate the type I error, and \( 10^5 \) experiments to evaluate the power under various combinations of \( (\gamma_1, \ldots, \gamma_m) \). We conducted simulations for \( p_0 = (0.1, 0.3), p_1 = 0.1, \) and \( \rho = 0, 0.2, 0.5, 0.8 \). Here we report the results for \( m = 2, 8, \rho = 0, 0.5 \) and \( p_0 = 0.1 \). The conclusions remain the same for other settings (data not shown).
Table 3: Type I error of testing two continuous traits, scaled by the nominal significance level $\alpha$. The MAFs of SNP are 0.1 and 0.2 in the two populations. $Q$ is the $m$-DF omnibus Wald test, $T$ and $T'$ are the 1-DF Wald tests assuming a common or common scaled effect. $(Q_s, T_s, T'_s)$ are the corresponding MLM GEE based $m$-DF omnibus test and 1-DF tests assuming a common effect or common scaled effect. $(\tilde{Q}, \tilde{T}, \tilde{T}')$ are the Wald tests using chi-square distribution to compute p-values.

| $\alpha$ | $Q$ | $T$ | $T'$ | $Q$ | $T$ | $T'$ | $Q_s$ | $T_s$ | $T'_s$ |
|----------|-----|-----|------|-----|-----|------|-------|-------|-------|
| $10^{-5}$ | 1.03 | 1.06 | 1.08 | 1.19 | 1.16 | 1.18 | 0.69  | 0.86  | 0.87  |
| $10^{-4}$ | 1.02 | 1.02 | 1.03 | 1.11 | 1.11 | 1.12 | 0.81  | 0.85  | 0.89  |
| $10^{-3}$ | 1.00 | 1.00 | 1.01 | 1.05 | 1.04 | 1.06 | 0.89  | 0.94  | 0.94  |

| $\rho = 0.5$ |
|--------------|
| $\alpha$     | $Q$ | $T$ | $T'$ | $Q$ | $T$ | $T'$ | $Q_s$ | $T_s$ | $T'_s$ |
|--------------|
| $10^{-5}$    | 1.07 | 0.96 | 1.06 | 1.15 | 1.08 | 1.10 | 0.66  | 0.66  | 0.83  |
| $10^{-4}$    | 1.04 | 1.00 | 1.00 | 1.14 | 1.08 | 1.07 | 0.82  | 0.87  | 0.91  |
| $10^{-3}$    | 0.99 | 0.99 | 1.00 | 1.05 | 1.03 | 1.04 | 0.90  | 0.93  | 0.93  |

Tables 3 and 4 summarize the estimated type I errors. Overall the type I errors are well controlled for the proposed methods, while the GEE score tests are conservative especially for large number of traits ($m = 8$).

Tables 5 and 6 summarize the power for $m = 2$ and $m = 8$ respectively. $T$ is the most powerful when $\gamma_j$ are close to each other, and $T'$ is the most powerful when $\gamma_j/\sigma_j$ are close to each other. In general the proposed MLM based Wald tests perform better than the corresponding GEE based score tests. This agrees with the general principle that the Wald test is typically more powerful than the GEE based test.
Table 4: Type I error of testing eight continuous traits, scaled by the nominal significance level $\alpha$. The MAFs of SNP are 0.1 and 0.2 in the two populations.

| $\alpha$ | $Q$  | $T$  | $T'$ | $Q$  | $T$  | $T'$ | $Q_s$ | $T_s$ | $T'_s$ |
|---------|------|------|------|------|------|------|-------|-------|-------|
| $10^{-5}$ | 0.84 | 1.07 | 1.05 | 1.11 | 1.25 | 1.15 | 0.48  | 0.85  | 0.78  |
| $10^{-4}$ | 0.88 | 0.99 | 1.00 | 1.11 | 1.10 | 1.11 | 0.60  | 0.86  | 0.85  |
| $10^{-3}$ | 0.93 | 1.01 | 1.00 | 1.11 | 1.08 | 1.09 | 0.75  | 0.95  | 0.94  |

$\rho = 0, p_0 = 0.1$

| $\alpha$ | $Q$  | $T$  | $T'$ | $Q$  | $T$  | $T'$ | $Q_s$ | $T_s$ | $T'_s$ |
|---------|------|------|------|------|------|------|-------|-------|-------|
| $10^{-5}$ | 0.92 | 0.78 | 0.93 | 1.33 | 0.98 | 1.05 | 0.42  | 0.62  | 0.65  |
| $10^{-4}$ | 0.94 | 0.96 | 0.99 | 1.24 | 1.09 | 1.12 | 0.64  | 0.90  | 0.86  |
| $10^{-3}$ | 0.95 | 0.95 | 1.00 | 1.13 | 1.02 | 1.07 | 0.76  | 0.94  | 0.94  |

$\rho = 0.5, p_0 = 0.1$

| $\alpha$ | $Q$  | $T$  | $T'$ | $Q$  | $T$  | $T'$ | $Q_s$ | $T_s$ | $T'_s$ |
|---------|------|------|------|------|------|------|-------|-------|-------|
| $10^{-5}$ | 1.00 | 0.96 | 1.03 | 1.42 | 1.15 | 1.17 | 0.66  | 0.86  | 0.92  |
| $10^{-4}$ | 0.90 | 0.96 | 0.97 | 1.17 | 1.07 | 1.10 | 0.77  | 0.90  | 0.90  |
| $10^{-3}$ | 0.93 | 1.01 | 1.02 | 1.11 | 1.09 | 1.10 | 0.84  | 0.97  | 0.97  |

$\rho = 0, p_0 = 0.3$

| $\alpha$ | $Q$  | $T$  | $T'$ | $Q$  | $T$  | $T'$ | $Q_s$ | $T_s$ | $T'_s$ |
|---------|------|------|------|------|------|------|-------|-------|-------|
| $10^{-5}$ | 0.80 | 0.82 | 0.87 | 1.28 | 1.01 | 1.05 | 0.47  | 0.75  | 0.72  |
| $10^{-4}$ | 0.91 | 0.88 | 1.00 | 1.16 | 0.99 | 1.15 | 0.73  | 0.88  | 0.95  |
| $10^{-3}$ | 0.92 | 0.94 | 1.00 | 1.09 | 1.01 | 1.06 | 0.84  | 0.97  | 0.97  |

$\rho = 0.5, p_0 = 0.3$
Table 5: Power of multi-trait tests for $m = 2$ continuous traits ($Y_1, Y_2$) under significance level $\alpha = 10^{-4}$. The MAFs of SNP are 0.1 and 0.2 in the two populations respectively. $Q$ is the $m$-DF omnibus Wald test, $T$ and $T'$ are the 1-DF Wald tests assuming common or common scaled effect. ($Q_s, T_s, T'_s$) are the corresponding GEE based $m$-DF omnibus test and 1-DF tests assuming a common effect or common scaled effect. $\sigma_i$ is the standard error of $Y_i$ and $\gamma_i$ is the SNP coefficient, $i = 1, 2$. The highest powered tests are bold-faced.

| $(\gamma_1, \gamma_2)$ | $(\frac{\sigma_1}{\gamma_1}, \frac{\sigma_2}{\gamma_2})$ | $Q$  | $T$  | $T'$ | $Q_s$ | $T_s$ | $T'_s$ |
|-------------------------|-----------------|-------|-------|-------|-------|-------|-------|
| $(0.3,0)$               | $(0.21,0)$      | **0.205** | 0.025 | 0.064 | 0.178 | 0.020 | 0.052 |
| $(0.3,0.1)$             | $(0.21,0.1)$    | 0.316 | 0.249 | **0.337** | 0.278 | 0.217 | 0.302 |
| $(0.25,0.18)$           | $(0.18,0.18)$   | 0.418 | 0.509 | **0.530** | 0.374 | 0.470 | 0.494 |
| $(0.3,0.25)$            | $(0.21,0.25)$   | 0.831 | 0.891 | **0.892** | 0.796 | 0.869 | 0.870 |
| $(0.2,0.2)$             | $(0.14,0.2)$    | 0.376 | **0.484** | 0.462 | 0.335 | 0.449 | 0.426 |
| $(0.2,0.25)$            | $(0.14,0.25)$   | 0.631 | **0.727** | 0.676 | 0.585 | 0.694 | 0.638 |
| $(0.25,0.25)$           | $(0.18,0.25)$   | 0.731 | **0.818** | 0.799 | 0.690 | 0.791 | 0.769 |
| $(0.0,25)$              | $(0.0,25)$      | **0.401** | 0.247 | 0.133 | 0.359 | 0.216 | 0.108 |
| $(0.0,3)$               | $(0.0,3)$       | **0.701** | 0.486 | 0.291 | 0.657 | 0.439 | 0.239 |
| $(0.1,0.25)$            | $(0.07,0.25)$   | 0.463 | **0.484** | 0.372 | 0.418 | 0.448 | 0.331 |
| $(0.1,0.3)$             | $(0.07,0.3)$    | **0.744** | 0.726 | 0.590 | 0.701 | 0.690 | 0.535 |
| $(0.2,0.3)$             | $(0.14,0.3)$    | 0.842 | **0.890** | 0.842 | 0.810 | 0.869 | 0.809 |

$\rho = 0.5$

| $(\gamma_1, \gamma_2)$ | $(\frac{\sigma_1}{\gamma_1}, \frac{\sigma_2}{\gamma_2})$ | $Q$  | $T$  | $T'$ | $Q_s$ | $T_s$ | $T'_s$ |
|-------------------------|-----------------|-------|-------|-------|-------|-------|-------|
| $(0.3,0)$               | $(0.21,0)$      | **0.377** | 0.001 | 0.025 | 0.334 | 0.001 | 0.019 |
| $(0.3,0.1)$             | $(0.21,0.1)$    | **0.208** | 0.049 | 0.145 | 0.179 | 0.041 | 0.127 |
| $(0.25,0.18)$           | $(0.18,0.18)$   | 0.178 | 0.218 | **0.255** | 0.153 | 0.192 | 0.232 |
| $(0.3,0.25)$            | $(0.21,0.25)$   | 0.522 | 0.615 | **0.617** | 0.477 | 0.573 | 0.582 |
| $(0.2,0.2)$             | $(0.14,0.2)$    | 0.175 | **0.255** | 0.214 | 0.151 | 0.229 | 0.192 |
| $(0.2,0.25)$            | $(0.14,0.25)$   | 0.408 | **0.498** | 0.364 | 0.366 | 0.464 | 0.331 |
| $(0.25,0.25)$           | $(0.18,0.25)$   | 0.448 | **0.558** | 0.493 | 0.403 | 0.521 | 0.457 |
| $(0.0,25)$              | $(0.0,25)$      | **0.639** | 0.277 | 0.052 | 0.590 | 0.247 | 0.404 |
| $(0.0,3)$               | $(0.0,3)$       | **0.890** | 0.525 | 0.120 | 0.863 | 0.476 | 0.094 |
| $(0.1,0.25)$            | $(0.07,0.25)$   | **0.451** | 0.383 | 0.165 | 0.405 | 0.354 | 0.141 |
| $(0.1,0.3)$             | $(0.07,0.3)$    | **0.771** | 0.640 | 0.300 | 0.730 | 0.607 | 0.257 |
| $(0.2,0.3)$             | $(0.14,0.3)$    | 0.703 | **0.746** | 0.548 | 0.659 | 0.718 | 0.504 |
Table 6: Power of multi-trait tests for $m = 8$ continuous traits under significance level $\alpha = 10^{-4}$. The MAFs of SNP are 0.1 and 0.2 in the two populations respectively. $Q$ is the $m$-DF omnibus Wald test, $T$ and $T'$ are the 1-DF Wald tests assuming common or common scaled effect. $(Q_s, T_s, T'_s)$ are the corresponding GEE based $m$-DF omnibus test and 1-DF tests assuming a common effect or common scaled effect. The highest powered tests are bold-faced.

| $\rho = 0$ | $\rho = 0.5$ |
|------------|-------------|
| $\gamma_1 = 0.3, \gamma_{i>1} = 0$ | 0.063 0.001 0.003 0.044 0.001 0.002 |
| (.3,.2,.1,.05,0,...,0) | 0.457 0.153 0.219 0.373 0.101 0.152 |
| $\gamma_1 = 0.2, \gamma_{i>1} = 0.15$ | 0.933 0.995 0.996 0.889 0.992 0.993 |
| $\gamma_i = 0.15$ | 0.908 0.994 0.993 0.855 0.990 0.989 |
| $\gamma_1 = 0.2, \gamma_{i>1} = 0.15$ | 0.297 0.001 0 0.230 0 0 |
| (.3,.2,.1,.05,0,...,0) | 0.688 0.001 0.008 0.596 0 0.005 |
| $\gamma_1 = 0.2, \gamma_{i>1} = 0.15$ | 0.043 0.196 0.217 0.030 0.169 0.198 |
| $\gamma_i = 0.15$ | 0.045 0.230 0.190 0.031 0.203 0.172 |

6 R package MTAR

We have implemented the proposed methods in an R package “MTAR” available at [http://www.github.com/baolinwu/MTAR](http://www.github.com/baolinwu/MTAR). The following lists some sample R codes to install and use the package.

```r
## Install MTAR
devtools::install_github("baolinwu/MTAR")

## Multi-trait association test
library(VGAM)
library(MTAR)
Z = rbinom(1000,1,0.5)
G = rbinom(1000,2,0.25)
## assume the same covariates
X = rnorm(1000)
```

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e = rnorm(1000)
Y1 = Z+X + 0.15*G + rnorm(1000)+e
Y2 = Z+X + 0.1*G + rnorm(1000)+e
Y = cbind(Y1,Y2)
obj = MLM.null(Y,cbind(Z,X))
MQTAc(obj, G)

## different covariates
X1 = rnorm(1000)
X2 = rnorm(1000)
Y1 = Z+X1 + 0.15*G + rnorm(1000)+e
Y2 = Z+X2 + 0.1*G + rnorm(1000)+e
Y = cbind(Y1,Y2)
YX = list(Y, cbind(X1,Z), cbind(X2,Z))
objd = MDM.null(YX,pux=2)
MQTAd(objd, G)

The developed algorithms are very efficient and extremely scalable to genome-wide association test. For example, it takes 30 minutes to conduct joint association tests for around 2.5 million HapMap SNPs in the ARIC data on a single Linux desktop with 3.0 GHz CPU and 24 GB memory. The eigen decompositions involved are computed very efficiently, since we just need to compute the top eigen vectors for the covariate matrix. The covariance matrix involved in the Wald tests has the same dimension as the number of traits, and its inverse can also be computed efficiently.
Table 7: Type I error (divided by the significance level $\alpha$) when simulating from $K$-DF multivariate t-distribution.

| $\alpha$ | $K=5$ | $K=10$ | $K=20$ |
|----------|-------|--------|--------|
|         | $Q$   | $T'$   | $T''$  | $Q$    | $T'$   | $T''$  | $Q$    | $T'$   | $T''$  |
| $10^{-4}$ | 0.92  | 0.97   | 0.95   | 0.92   | 0.84   | 0.82   | 0.92   | 0.87   | 0.87   |
| $10^{-3}$ | 0.95  | 0.98   | 0.98   | 1.01   | 0.99   | 0.99   | 0.97   | 0.96   | 0.98   |
| $10^{-2}$ | 0.99  | 1.00   | 1.00   | 0.99   | 1.00   | 1.00   | 1.00   | 1.00   | 1.00   |

7 Discussions

We note that the multi-trait test approach generally benefits the most when the marginal effects have different directions from the trait correlations. For example, consider a bivariate normal random vector $Z = (z_1, z_2)^T$ with covariance matrix $\Sigma$ having unit variance and 0.5 correlation. Consider the Wald test $Z^T \Sigma^{-1} Z$. Under $5 \times 10^{-8}$ significance level, its test power is 0.5% with $E(z_1) = 3, E(z_2) = 2$, while the power is 24.8% when $E(z_1) = 3, E(z_2) = -2$.

The proposed Wald tests are generally robust to deviation from normality, partly because GWAS are based on large sample sizes, and the F-distribution based Wald test is robust. Here we conduct a simple simulation study to investigate the type I errors of the proposed Wald tests when we simulate the outcomes from the multivariate t-distribution instead. We consider 5000 individuals with two outcomes following $K$-DF bivariate t-distribution with unit variance and 0.5 correlation. The genotype is simulated from Binom(2, 0.2). We conducted $10^6$ simulations to investigate the type I errors at significance levels $\alpha = 10^{-2, -3, -4}$ under $K = 5, 10, 20$. Table 7 summarizes the results. Overall we can see that all proposed Wald tests have well controlled type I errors.

GWAS are typically useful to identify common variants (MAF $\geq$ 5%). The proposed Wald tests are applicable to any MAF and number of traits, in the sense that the Wald test F-distributions always hold. However we do recommend that the tests are only applied to
common variants and relatively small number of traits: for PheWAS with huge number of traits (Pendergrass et al., 2011), more efforts are needed to develop new and powerful tests.

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