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A Family-Based Robust Multivariate Association Test Using Maximum Statistic

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Summary

For characterizing the genetic mechanisms of complex diseases familial data with multiple correlated quantitative traits are usually collected in genetic studies. To analyze such data, various multivariate tests have been proposed to investigate the association between the underlying disease genes and the multiple traits. Although these multivariate association tests may have better power performance than the univariate association tests, they suffer from loss of testing power when the genetic models of the putative genes are misspecified. To address the problem, in this paper we aim to develop a family-based robust multivariate association test. We will first establish the optimal multivariate score tests for the recessive, additive, and dominant genetic models. Based on these optimal tests, a maximum-type robust multivariate association test is then obtained. Simulations are conducted to compare the power of our method with that of other existing multivariate methods. The results show that the robust multivariate test does manifest the robustness in power over all plausible genetic models. A practical data set is applied to demonstrate the applicability of our approach. The results suggest that the robust multivariate test is more powerful than the robust univariate test when dealing with multiple quantitative traits.

Keywords: Candidate gene, copula, genetic model, multiple quantitative traits

Introduction

Due to the tremendous availability of genetic variants, genome-wide association studies have become a useful instrument for identification of genes underlying complex diseases in recent years (Diabetes Genetics Initiative of Broad Institute of Harvard and MIT et al., 2007; Stefansson et al., 2009). Because most complex diseases are measured in continuous scale or so-called "quantitative traits," the need to develop methods for discovering the underlying loci of quantitative traits has attracted the attention of researchers in modern human genetics. On the basis of familial data, the quantitative trait loci (QTLs) can be detected via investigation of the association between a quantitative trait and a marker locus (Allison, 1997; Rabinowitz, 1997; Abecasis et al., 2000; Monks & Kaplan, 2000; Kistner & Weinberg, 2004; Wheeler & Cordell, 2007; Guo & Shugart, 2012; Liu & Leal, 2012). Such family-based association approaches have the merit that they not only extract the required inheritance information but also avoid spurious association results caused by population stratification. However, since these methods often assume that the true underlying genetic model (mode of inheritance) of a putative locus is additive, they may incur a substantial loss of power when the true underlying genetic model is misspecified in practical studies. To deal with this problem, over the past decade, many population- and family-based robust association analysis methods have been widely proposed in the literature for investigating binary disease traits (Gastwirth & Freidlin, 2000; Zheng et al., 2002; Zheng & Tian, 2006; Yuan et al., 2009; Joo et al., 2010), though relatively fewer research studies have been carried out in this regard for quantitative traits (Wang & Tai, 2009; So & Sham, 2011).

Theoretically, in genetic association analysis, the use of multiple quantitative traits should be able to extract more information than use of a single trait (Liu et al., 2008; Melton et al., 2010). Besides, it should be noted that because the collected traits in a study may be affected simultaneously by the target QTL and the common environment which they share, correlations could exist among them. To analyze such

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multiple correlated quantitative trait data, a useful method is to conduct separate analysis for each quantitative trait, and then to draw conclusion from these multiple analysis results. However, such a univariate analysis approach would suffer from the problem of multiple comparisons and may lead to the potential loss of power when neglecting the correlations among multiple quantitative traits. An alternative method is to adopt the multivariate analysis approach to enhance the power (Lange et al., 2003; Klei et al., 2008; Zhang et al., 2010; Zhu et al., 2012). Since these methods also ignore the misspecification problem in genetic models, in this paper we are interested in the development of a robust multivariate association analysis method to address this issue.

In the Materials and Methods section, we will first demonstrate how to extract the association information between a marker and multiple quantitative traits through the conditional likelihood (Schaid & Sommer, 1993; Kistner & Weinberg, 2004; Wheeler & Cordell, 2007; Wang & Tai, 2009). We will establish three optimal multivariate score tests under the recessive, additive, and dominant models, respectively. We will combine the three tests to construct a robust association test statistic. The robust testing method was originally proposed by Davies (1977) and has been widely applied to genetic studies (Zheng, 2004; Zheng & Chen, 2005; González et al., 2008; Li et al., 2008b; Zang et al., 2010). In the Results section, we conduct simulation studies to compare the proposed robust multivariate association test with the optimal multivariate score tests under the correct and incorrect genetic models and with other existing multivariate association tests on type I error rates and testing powers. In addition, the GAW 14 Collaborative Study on the Genetics of Alcoholism (COGA) data are used to demonstrate the applicability of our proposed robust multivariate association test. Finally, we will conclude our studies with the Discussion section.

Materials and Methods

Data Structure

A random sample of n parent–offspring nuclear families is collected to extract the information of association between a marker and a set of quantitative traits. In each family, the two parents and their offspring are genotyped for the marker, and the multiple quantitative traits of the offspring are measured. The data of nuclear families can be restructured to become the data of triad families by dividing the families with multiple offspring into a number of single offspring families (Laird et al., 2000). If pedigrees are also included in the sample, the same dividing operation can be applied to yield simple triad data as well. However, triad data obtained by this restructuring procedure would lead to the situation that triad families separated from the same nuclear family (or pedigree) cluster together and thus the quantitative traits of the offspring in a cluster should be correlated. In general, analysis of such clustered data should take the within-cluster correlation into consideration, but in the following discussion, since a conditional likelihood-based analysis approach is adopted under the scenario that the offsprings' QTL genotypes are independently inherited and the quantitative traits of the offspring in a cluster are assumed conditionally independent on their QTL genotypes, the effect of within-cluster correlation is ignored. Note that if the restructured triad data are used in practice, the analysis procedure would be somewhat similar to a method which assigns weights to different clusters. Clusters of a greater number of divided triads are assigned higher weights. For simplicity, we will assume that there is merely one offspring in each collected family to illustrate our method in the following investigation. Consider the situation that the marker locus has two alleles A and a with frequencies p and q, respectively, where A labels the mutant allele and a the normal allele and $p + q = 1$. For a sample of n triad families, 10 categories can be classified based on the parental mating type and the offspring genotype (Table 1). Following Wang & Tai (2009), denote the ith parental mating type by $g_p = i$, $i = 1, \ldots, 6$, the jth offspring genotype by $g_c = j$, $j = 0, 1, 2$, and the m quantitative traits of the kth offspring member of the category $(g_p = i, g_c = j)$ by $\mathbf{y}_{ijk} = (y_{ijk}^{(1)}, \ldots, y_{ijk}^{(r)}, \ldots, y_{ijk}^{(m)})^T$, where $y_{ijk}^{(r)}$ is the rth quantitative trait of the offspring in the kth triad family of the category $(g_p = i, g_c = j)$, $r = 1, \ldots, m$, $k = 1, \ldots, n_{ij}$, and n_{ij} is the number of families in the category $(g_p = i, g_c = j)$.

Construction of Conditional Likelihood of Parent–Offspring Triad Families

Using case-parent triad families and conditional on the parental mating type and the affected offspring, Schaid & Sommer (1993) proposed a likelihood approach for assessing the association between a marker and a binary disease trait. Such an approach has the advantage that it is free from the necessity of assuming Hardy–Weinberg equilibrium for the marker locus, and from the result of spurious association due to population stratification. In the light of this conditional idea, two extended methods had been further developed for investigating the association between a marker and a quantitative trait (Kistner & Weinberg, 2004; Wheeler & Cordell, 2007). A robust quantitative trait association approach for handling the problem where the mode of inheritance of the investigated QTL is unknown was also proposed (Wang & Tai, 2009). In the following discussion, the method of Wang

Table 1 Classification of n parent–offspring triad families in terms of the parental mating types and the offspring genotypes.

Parental mating types (g_p)	Offspring genotypes (g_c)	Offspring multiple quantitative traits ($\mathbf{y}_{ijk}$)	Number of triad families (n_{ij})
$AA \times AA (i = 1)$	$AA (j = 2)$	$\mathbf{y}_{121}, \ldots, \mathbf{y}_{12n_{12}}$	n_{12}
$AA \times Aa (i = 2)$	$AA (j = 2)$	$\mathbf{y}_{221}, \ldots, \mathbf{y}_{22n_{22}}$	n_{22}
	$Aa (j = 1)$	$\mathbf{y}_{211}, \ldots, \mathbf{y}_{21n_{21}}$	n_{21}
$AA \times aa (i = 3)$	$Aa (j = 1)$	$\mathbf{y}_{311}, \ldots, \mathbf{y}_{31n_{31}}$	n_{31}
$Aa \times Aa (i = 4)$.	$AA (j = 2)$	$\mathbf{y}_{421}, \ldots, \mathbf{y}_{42n_{42}}$	n_{42}
	$Aa (j = 1)$	$\mathbf{y}_{411}, \ldots, \mathbf{y}_{41n_{41}}$	n_{41}
	$aa (j = 0)$	$\mathbf{y}_{401}, \ldots, \mathbf{y}_{40n_{40}}$	n_{40}
$Aa \times aa (i = 5)$	$Aa (j = 1)$	$\mathbf{y}_{511}, \ldots, \mathbf{y}_{51n_{51}}$	n_{51}
	$aa (j = 0)$	$\mathbf{y}_{501}, \ldots, \mathbf{y}_{50n_{50}}$	n_{50}
$aa \times aa (i = 6)$	$aa (j = 0)$	$\mathbf{y}_{601}, \ldots, \mathbf{y}_{60n_{60}}$	n_{60}

& Tai (2009) is extended to allow for assessing the association between a marker and multiple quantitative traits. The conditional probabilities of observing the offspring genotype $g_c = j$ given the parental mating type $g_p = i$ and the multiple quantitative traits $\mathbf{y}_{ijk}$ for an offspring member k in the category $(g_p = i,\ g_c = j)$ as shown in Table 1 can be expressed as:

$$P(g_c = j \mid g_p = i, \mathbf{y}_{ijk}) = \frac{P(\mathbf{y}_{ijk} \mid g_c = j) P(g_c = j \mid g_p = i)}{\sum_{j'} P(\mathbf{y}_{ijk} \mid g_c = j') P(g_c = j' \mid g_p = i)}. \quad (1)$$

Note that in the above setting we have assumed $P(\mathbf{y}_{ijk}|g_c = j, g_p = i) = P(\mathbf{y}_{ijk}|g_c = j)$. That is, given that the offspring's genotype is available, we assume that the parental genotype can provide no more information for measuring the probability of the traits $\mathbf{y}_{ijk}$. Assume also that the genotyped bi-allelic marker locus is indeed the QTL responsible for the variations of the collected multiple quantitative traits. This marker is also referred to as the candidate gene of these quantitative traits. The quantities of the three marker genotypes AA, Aa, and aa corresponding to the m traits could be different and they are denoted by three vectors: $\mathbf{u}_2$, $\mathbf{u}_1$, and $\mathbf{u}_0$, where $\mathbf{u}_j = (u_{1j}, \ldots, u_{mj})^T$, $j = 2(AA), 1(Aa), 0(aa)$. Let the vector $\mathbf{d} = (d_1, \ldots, d_m)^T$ be the displacement effects between the genotypes AA and aa for the m traits and let t be the common degree of dominance of the QTL. The genotypic values $\mathbf{u}_2$ and $\mathbf{u}_1$ can be reparameterized by $\mathbf{d}$, t, and $\mathbf{u}_0$, as $\mathbf{u}_2 = \mathbf{u}_0 + \mathbf{d}$ and $\mathbf{u}_1 = \mathbf{u}_0 + t\mathbf{d}$. Note that here the degrees of dominance of the QTL for the m traits are set as a constant, which means that the modes of inheritance of the QTL corresponding to the m different traits are assumed to be identical. The value of t would correspond to 0, 1/2, or 1, respectively, if the underlying genetic model of the QTL is recessive, additive, or dominant. These three typical genetic models are chosen for deriving three optimal multivariate score tests in the next subsection. Theoretically, all the elements of the displacement effects in $\mathbf{d}$ should be greater than zero, if the test locus is indeed the QTL of the measured traits. Accordingly, testing whether or not $\mathbf{d} = \mathbf{0}$ is equivalent to testing whether there is any association between the marker and any one of the measured traits. That is, the displacement effects are absent for the m quantitative traits (viz., $\mathbf{u}_2 = \mathbf{u}_1 = \mathbf{u}_0$). It should be noted that when testing the displacement effects $\mathbf{d}$, the degree of dominance t plays a role of nuisance parameter, which is absent under the null hypothesis, but is present under the alternative hypothesis (Davies, 1977).

To implement the conditional inference procedure using parent–offspring triad families, we first have to formulate the conditional distribution of the multiple quantitative traits $\mathbf{y}_{ijk}$ in (1). Under the assumption that the sampled multiple quantitative traits are influenced by a target QTL and environmental factors and there is no interaction between them, the multiple quantitative traits of an offspring with the QTL genotype $g_c = j$ can be expressed as $\mathbf{y}_{ijk} = \mathbf{u}_j + \mathbf{e}_{ijk}$, where $e_{ijk} = (e_{ijk}^{(1)}, \ldots, e_{ijk}^{(m)})^T$ are the m environmental factors corresponding to the m traits. Assume that the environmental factors $\mathbf{e}_{ijk}$ of all offspring members are jointly distributed as a multivariate normal distribution with mean vector $\mathbf{0}$ and $m \times m$ environmental variance–covariance matrix $\mathbf{\Sigma}$, then the multiple quantitative traits $\mathbf{y}_{ijk}$ of an offspring with the QTL genotype $g_c = j$ are jointly distributed as a multivariate normal distribution with mean vector $\mathbf{u}_j$ and $m \times m$ variance–covariance matrix $\mathbf{\Sigma}$. Therefore, the probability density function $P(\mathbf{y}_{ijk}|g_c = j)$ in (1) is formulated as

$$P(\mathbf{y}_{ijk} \mid g_c = j) = (2\pi)^{-\frac{m}{2}} |\mathbf{\Sigma}|^{-\frac{1}{2}} \exp\left[-\frac{\left(\mathbf{y}_{ijk} - \mathbf{u}_j\right)^T \mathbf{\Sigma}^{-1} \left(\mathbf{y}_{ijk} - \mathbf{u}_j\right)}{2} \right], \quad (2)$$

which implies that the multiple quantitative traits of all offspring members in the population have the same distribution form but have different mean vectors according to their

corresponding genotypes. Because the offspring members of all triad families are independent under the random sampling procedure, and $\mathbf{u}_2$ and $\mathbf{u}_1$ are the functions of the parameters $\mathbf{d}$, t, and $\mathbf{u}_0$, based on the conditional probability in (1) and the probability density function in (2), a conditional likelihood for test of $H_0 : \mathbf{d} = \mathbf{0}$ is further established as

$$L(\mathbf{d}, t, \mathbf{u}_0; \mathbf{y}) = \prod_{i,j}\prod_{k=1}^{n_{ij}} \frac{(2\pi)^{-\frac{m}{2}}|\boldsymbol{\Sigma}|^{-\frac{1}{2}}\exp\left[-\frac{\left(\mathbf{y}_{ijk}-\mathbf{u}_j\right)^T\boldsymbol{\Sigma}^{-1}\left(\mathbf{y}_{ijk}-\mathbf{u}_j\right)}{2}\right]\cdot p_{j|i}}{\sum_{j'}(2\pi)^{-\frac{m}{2}}|\boldsymbol{\Sigma}|^{-\frac{1}{2}}\exp\left[-\frac{\left(\mathbf{y}_{ijk}-\mathbf{u}_{j'}\right)^T\boldsymbol{\Sigma}^{-1}\left(\mathbf{y}_{ijk}-\mathbf{u}_{j'}\right)}{2}\right]\cdot p_{j'|i}}, \quad (3)$$

where $p_{j|i}$ denotes the transmission probability $P(g_c = j|g_p = i)$.

The Optimal Multivariate Score Tests for Recessive, Additive, and Dominant Models

In this subsection, we will derive the optimal multivariate score test statistics corresponding to the recessive, additive, and dominant models. Let vector $\mathbf{U}_{H_0} = (U_{H_0}^{(1)}, \ldots, U_{H_0}^{(m)})^T$ be the m-dimensional score statistics under the null hypothesis $H_0 : \mathbf{d} = \mathbf{0}$ and $\mathbf{I}_{H_0} = (Cov(U_{H_0}^{(\ell)}, U_{H_0}^{(\ell')}))$ be the $m \times m$ variance–covariance matrix of $\mathbf{U}_{H_0}$, ℓ and $\ell' = 1, \ldots, m$. The ℓth score statistic of $\mathbf{U}_{H_0}$ (see the Appendix in Supporting Information) is obtained as

$$U_{H_0}^{(\ell)} = \sum_{i,j}\sum_{k=1}^{n_{ij}}\left[\left(\mathbf{y}_{ijk}-\boldsymbol{\mu}\right)^T\boldsymbol{\Sigma}_{.\ell}^{-1}\right]\left\{D_{\ell j} - E_i(D_\ell)\right\}, \quad (4)$$

where $\boldsymbol{\mu}$ is the population mean vector for the m quantitative traits, $\boldsymbol{\Sigma}_{.\ell}^{-1}$ is the ℓth column of the inverse of the variance–covariance matrix $\boldsymbol{\Sigma}$, $D_{\ell j} = \partial u_{\ell j}/\partial d_\ell$ is the first derivative of the ℓth genotypic value $u_{\ell j}$ of the vector $\mathbf{u}_j$ with respect to the ℓth displacement effect d_ℓ of the vector $\mathbf{d}$ for $j = 0, 1, 2$ and $\ell = 1, \ldots, m$ such that $D_{\ell 0} = 0$, $D_{\ell 1} = t$ and $D_{\ell 2} = 1$, and $E_i(D_\ell) = \sum_{j'=0}^{2} D_{\ell j'} p_{j'|i}$ is the conditional expectation of the first derivative D_ℓ on the ith parental mating type, $i = 1, \ldots, 6$. Note that since $E[D_{\ell j} - E_i(D_\ell)]$ in (4) is 0, the expected value of the score statistic $U_{H_0}^{(\ell)}$ is also 0, that is, $E(U_{H_0}^{(\ell)}) = 0$. Moreover, the covariance between any two score statistics $U_{H_0}^{(\ell)}$ and $U_{H_0}^{(\ell')}$ is calculated as

$$Cov\left(U_{H_0}^{(\ell)}, U_{H_0}^{(\ell')}\right) = \sum_{i,j}\sum_{k=1}^{n_{ij}}\left[\left(\mathbf{y}_{ijk}-\boldsymbol{\mu}\right)^T\boldsymbol{\Sigma}_{.\ell}^{-1}\right] \times \left[\left(\mathbf{y}_{ijk}-\boldsymbol{\mu}\right)^T\boldsymbol{\Sigma}_{.\ell'}^{-1}\right] Cov_i(D_\ell, D_{\ell'}), \quad (5)$$

ℓ and $\ell' = 1, \ldots, m$, where $Cov_i(D_\ell, D_{\ell'})$ is the conditional covariance between any two first derivatives D_ℓ and $D_{\ell'}$ on the ith parental mating type.

The estimates of $U_{H_0}^{(\ell)}$ and $Cov(U_{H_0}^{(\ell)}, U_{H_0}^{(\ell')})$ can be obtained by replacing the overall population mean vector $\boldsymbol{\mu}$ and the population variance–covariance matrix $\boldsymbol{\Sigma}$ of the m traits in these statistics with the sample mean vector $\bar{\mathbf{y}} = \frac{1}{n}\sum_{i,j,k}\mathbf{y}_{ijk}$ and the sample variance–covariance matrix $\mathbf{S} = \frac{1}{n-1}\sum_{i,j,k}(\mathbf{y}_{ijk}-\bar{\mathbf{y}})(\mathbf{y}_{ijk}-\bar{\mathbf{y}})^T$ such that the estimated score vector is $\hat{\mathbf{U}}_{H_0} = (\hat{U}_{H_0}^{(1)}, \ldots, \hat{U}_{H_0}^{(m)})^T$ and the estimated variance–covariance matrix of $\mathbf{U}_{H_0}$ is $\hat{\mathbf{I}}_{H_0} = (\hat{Cov}(U_{H_0}^{(\ell)}, U_{H_0}^{(\ell')}))$, ℓ and $\ell' = 1, \ldots, m$. The multivariate score test statistic for test of association between a marker gene and the m quantitative traits is constructed as $X^2 = \hat{\mathbf{U}}_{H_0}^T\hat{\mathbf{I}}_{H_0}^{-1}\hat{\mathbf{U}}_{H_0}$, which is asymptotically distributed as a χ^2 distribution with degrees of freedom ν, $\nu = rank(\hat{\mathbf{I}}_{H_0}) \leq m$ (Lange & Laird, 2002). If the m quantitative traits are linear and independent, then $\nu = m$ (Lange et al., 2003). By setting $t = 0$ (recessive), 1/2 (additive), and 1 (dominant), the three corresponding multivariate score test statistic X^2 are further obtained as $X_{REC}^2 = \hat{\mathbf{U}}_{REC}^T\hat{\mathbf{I}}_{REC}^{-1}\hat{\mathbf{U}}_{REC}$, $X_{ADD}^2 = \hat{\mathbf{U}}_{ADD}^T\hat{\mathbf{I}}_{ADD}^{-1}\hat{\mathbf{U}}_{ADD}$, and $X_{DOM}^2 = \hat{\mathbf{U}}_{DOM}^T\hat{\mathbf{I}}_{DOM}^{-1}\hat{\mathbf{U}}_{DOM}$, respectively (see the Appendix in Supporting Information). These three optimal multivariate score test statistics X_{REC}^2, X_{ADD}^2, and X_{DOM}^2 are employed for constructing a robust multivariate association test statistic in the following subsection.

Robust Multivariate Association Test

When the genetic model of the investigated quantitative trait cannot be identified in practical studies, developing a robust multivariate association test that can deal with uncertainty is useful. Here, we adopt the maximum statistic procedure to construct such a method (Davies, 1977). It is noted that hypothesis testing with a nuisance parameter can be addressed by use of the union-intersection test (Casella & Berger, 2002); the maximum test statistic is robust against the nuisance parameter due to the fact that it is indeed the optimal test statistic in the union-intersection test. In dealing with the model uncertainty problem here, if there is absence of either overdominance or underdominance of the QTL, the nuisance parameter t (degree of dominance) ranges from 0 to 1 (recessive to dominant model). Previous studies on coping with this problem had utilized the maximum of the score test statistics at the two extreme genetic models (i.e., X_{REC}^2 and X_{DOM}^2) and an additional intermediate genetic model (i.e., X_{ADD}^2) for establishment of a robust test statistic (Zheng et al., 2002; Zheng & Chen, 2005; Wang & Tai, 2009). In analogy to their approaches, here the robust multivariate association test statistic is set up as

$$MAX_m = \max\left\{X_{REC}^2, X_{ADD}^2, X_{DOM}^2\right\}.$$

The asymptotic distribution of the test statistic MAX_m is generally unavailable, a resampling-based procedure is adopted to simulate the null distribution of MAX_m. Recall that under the null hypothesis the displacement effect $\mathbf{d}$ is equal to $\mathbf{0}$, so the multiple quantitative traits of offspring members given their genotypes are all distributed as the multivariate normal distribution with the common mean vector $\boldsymbol{\mu}$ and the variance–covariance matrix $\boldsymbol{\Sigma}$. In simulation, based on a random mating assumption for a designated frequency p of allele A, the parental genotypes of a triad family can be generated and the offspring genotypes are randomly assigned according to Mendelian transmission. The corresponding multiple quantitative traits of the offspring will be produced by the multivariate normal distribution with an assigned mean vector $\bar{\mathbf{y}}$ and variance–covariance matrix $\mathbf{S}$. By generating a sufficiently large number B of null samples, the robust multivariate association test statistic for the bth replicate of the B null samples is obtained as $MAX_{\text{null}}^{(b)} = \max\{X_{REC,\text{null}}^{2(b)}, X_{ADD,\text{null}}^{2(b)}, X_{DOM,\text{null}}^{2(b)}\}$. The proportion of the $MAX_{\text{null}}^{(b)}$s that exceeds the observed test statistic $M\hat{A}X_m$ is the P-value of MAX_m, which is denoted by $P(MAX_m \geq M\hat{A}X_m|H_0) \approx \frac{1}{B}\sum_{b=1}^{B} I(MAX_{\text{null}}^{(b)} \geq M\hat{A}X_m)$.

In the real analysis, if the degree of dominance t can be narrowed down to a restricted extent through the prior knowledge of molecular biology (Schaid & Sommer, 1994), the robust statistic MAX_m can be further modified to be of higher power. For example, if previous studies supported the conjecture that the dominant model is reasonably excluded, then the MAX_m should be modified as $MAX_{RA} = \max\{X_{REC}^2, X_{ADD}^2\}$. Moreover, if the recessive model is fairly excluded, then the $MAX_{AD} = \max\{X_{ADD}^2, X_{DOM}^2\}$ can be considered. Comparison and assessment of robustness and power performances among the aforementioned multivariate test statistics are presented in the following section.

Results

Simulation Studies

To assess the robustness and power performances of our proposed robust multivariate association test, two existing tests, the multivariate family-based association test (FBAT-GEE) proposed by Lange et al. (2003) and the nonparametric generalized Kendall's tau-based association test (FBAT-Tau) proposed by Zhang et al. (2010), were included for comparison. Overall, the three optimal multivariate score tests X_{REC}^2, X_{ADD}^2, and X_{DOM}^2, the three robust multivariate tests MAX_m, MAX_{RA}, and MAX_{AD}, and the FBAT-GEE and FBAT-Tau were investigated. Parent–offspring triad families were generated to implement the comparisons. In each family, the genotypes of a bi-allelic QTL (A, a) of the parents and the offspring and the two investigated quantitative traits of the offspring were generated. Let $y^{(1)}$ and $y^{(2)}$ be the two quantitative traits and $\mathbf{y} = (y^{(1)}, y^{(2)})^T$. The environmental factors which are involved in the mechanism of $y^{(1)}$ and $y^{(2)}$ are denoted by $\mathbf{e} = (e^{(1)}, e^{(2)})^T$. We assume that the two quantitative traits were influenced by a common bi-allelic QTL and environmental factors $\mathbf{e}$ and there is no interaction between them. Let vectors $\mathbf{u}_2 = (u_{10} + d_1, u_{20} + d_2)^T$, $\mathbf{u}_1 = (u_{10} + td_1, u_{20} + td_2)^T$, and $\mathbf{u}_0 = (u_{10}, u_{20})^T$ be the genotypic values of AA, Aa, and aa of the QTL corresponding to $\mathbf{y} = (y^{(1)}, y^{(2)})^T$, where d_1 and d_2 are the displacement effects between the genotypes AA and aa for the traits $y^{(1)}$ and $y^{(2)}$, respectively, and t is the common degree of dominance of the QTL for the two quantitative traits. Accordingly, conditional on the generated offspring genotype AA, Aa, or aa, the two quantitative traits of the offspring in a family were produced by the model $\mathbf{y} = \mathbf{u}_2 + \mathbf{e}$, $\mathbf{y} = \mathbf{u}_1 + \mathbf{e}$, or $\mathbf{y} = \mathbf{u}_0 + \mathbf{e}$.

Two scenarios were considered in the simulation studies. In the first scenario, we assumed that conditional on the generated offspring genotype AA, Aa, or aa, the two quantitative traits of the offspring in a family were produced by the bivariate normal distribution with mean vector $\mathbf{u}_2$, $\mathbf{u}_1$, or $\mathbf{u}_0$ and 2×2 variance–covariance matrix $\boldsymbol{\Sigma}$ in which the diagonal elements were set to 1 (viz., the variances of the two quantitative traits were assigned value 1) and the off-diagonal elements was set to 0.3 (viz., the correlation coefficient ρ between the two quantitative traits was assigned value 0.3). Without loss of generality, in the simulation both u_{10} and u_{20} were fixed with value 0. In the second scenario, we assumed that conditional on the generated offspring genotype AA, Aa, or aa, the two quantitative traits of the offspring in a family were produced by the bivariate gamma distribution with shape parameter vector $\mathbf{u}_2$, $\mathbf{u}_1$, or $\mathbf{u}_0$ and fixed scale parameter vector $\mathbf{1}$. Note that here the bivariate gamma distribution was generated by the Clayton copula with a preset correlation coefficient $\rho = 0.3$ (viz., the correlation between the two quantitative traits was assigned value 0.3; Nelsen, 2006). Because all the shape parameters in the bivariate gamma distribution should be greater than zero, both u_{10} and u_{20} were fixed with value 6 as an example. Characterizing the skewness of the simulated bivariate data under the second scenario can be carried out via the univariate assessments (Ferreira & Steel, 2007). Table S1 lists the calculated result for the two variables/traits $y^{(1)}$ and $y^{(2)}$ given each specific QTL genotype, which shows that the simulated data are asymmetric and particularly skewed to the right in each variable direction. The frequency of allele A of the QTL was set at 0.1, 0.2, or 0.5 and a Hardy–Weinberg equilibrium population was assumed.

Because the power performances are diverse among different genetic models, in order to ensure that the robust tests

Table 2 Comparisons of empirical type I error rates under the null hypothesis and power under the three true models among the three optimal multivariate score tests (X^2_{REC}, X^2_{ADD}, and X^2_{DOM}), the three robust multivariate association tests (MAX_m, MAX_{RA}, and MAX_{AD}) and the two existing multivariate methods (FBAT-GEE and FBAT-Tau), under the scenario that the two quantitative traits are distributed as bivariate normal with correlation coefficient 0.3. Allele frequency of the QTL is 0.1, 0.2, or 0.5, and sample size is 500.

Underlying genetic models	X^2_{REC}	X^2_{ADD}	X^2_{DOM}	MAX_m	MAX_{RA}	MAX_{AD}	FBAT-GEE	FBAT-Tau
$p = 0.1$								
Null	0.045	0.049	0.049	0.049	0.051	0.048	0.049	0.050
Recessive	0.803	0.409	0.058	0.731	0.748	–	0.408	0.205
Additive	0.139	0.795	0.771	0.743	0.731	0.796	0.800	0.784
Dominant	0.054	0.760	0.800	0.736	–	0.790	0.762	0.751
$p = 0.2$								
Null	0.047	0.047	0.047	0.046	0.047	0.046	0.050	0.051
Recessive	0.795	0.305	0.057	0.708	0.736	–	0.306	0.275
Additive	0.243	0.807	0.744	0.748	0.743	0.795	0.797	0.778
Dominant	0.056	0.753	0.831	0.763	–	0.815	0.740	0.725
$p = 0.5$								
Null	0.047	0.048	0.047	0.049	0.048	0.047	0.052	0.050
Recessive	0.796	0.541	0.063	0.708	0.751	–	0.550	0.534
Additive	0.540	0.796	0.540	0.734	0.754	0.754	0.799	0.780
Dominant	0.063	0.551	0.806	0.718	–	0.761	0.559	0.541

have a reasonable basis for comparison with other tests under the three genetic models, we chose the values of d_1 and d_2 to yield the testing powers of the three optimal multivariate score tests X^2_{REC}, X^2_{ADD}, and X^2_{DOM} at a level around 80%. For example, for the situation of allele A frequency $p = 0.1$ in the bivariate normal distribution, we considered $d_1 = 2.50$ and $d_2 = 3.70$ under the recessive model, $d_1 = 0.75$ and $d_2 = 0.80$ under the additive model, and $d_1 = 0.40$ and $d_2 = 0.42$ under the dominant model as depicted in Table S2. According to the above settings, the corresponding powers of the three optimal tests are 0.803, 0.795, and 0.800 for the three specific genetic models (Table 2). In the simulation, 10,000 replicates were yielded for evaluation of the empirical type I error rates and powers of all tests at a predetermined significance level 0.05. For all replicates, 500 triad families and $B = 1000$ null samples were generated in each replicate.

Comparison of Empirical Type I Error Rates and Powers of the Multivariate Association Tests

Table 2 lists the simulated results of empirical type I error rates and powers of all association tests when the allele A frequency of the QTL was fixed at p =0.1, 0.2, or 0.5 and the two quantitative traits were generated from the bivariate normal distribution (scenario 1). It is obvious that the empirical type I error rates of all association tests approximate the prescribed nominal significance level 0.05. From Table 2, we can see that each specific multivariate score test surpasses the other tests in power under the true corresponding genetic model, but would incur loss of power under an incorrect genetic model. For example, in the case of $p = 0.1$, X^2_{REC}, X^2_{ADD}, and X^2_{DOM}, respectively, manifest the best power performance at the levels of 0.803, 0.795, and 0.800 under the recessive, additive, and dominant models. However, the power of X^2_{REC} is down to the level of 0.139 under the additive model, and even down to the level of 0.054 under the dominant model. Unlike the three specific score tests, the robust multivariate association test MAX_m has relatively stable power performances over the three genetic models. For example, in Table 2 in the scenario of $p = 0.1$, all the powers of MAX_m achieve the level of 0.73 under the three genetic models. It is noted that X^2_{ADD} also exhibits robustness under the three genetic models, but compared with the MAX_m, the power performances of X^2_{ADD} are relatively low under the recessive model. The two existing multivariate methods, FBAT-GEE and FBAT-Tau have similar power performances as X^2_{ADD}. When the dominant or recessive model is reasonably excluded from our plausible genetic models, a modified robust multivariate association test MAX_{RA} or MAX_{AD} can be selected to replace the MAX_m to enhance the power of the test. For example, for $p = 0.1$ in Table 2, the power of MAX_{RA} is 0.748, which is notably greater than the power of MAX_m 0.731 under the recessive model. Similarly, it is shown that the power of MAX_{AD} is predominant over the MAX_m when the recessive model is reasonably excluded. For other allele A frequencies, $p = 0.2$ and $p = 0.5$, similar results in terms of power are observed in Table 2.

Table S3 lists the simulation results of empirical type I error rates and powers of all association tests when the frequency of allele A of the QTL was fixed at p =0.1, 0.2, or 0.5 and the two quantitative traits were generated from the bivariate gamma distribution (scenario 2). It can be seen that the empirical type I error rates of all association tests also approximate the significance level 0.05 and the robust multivariate association test MAX_m also exhibits its stable power performances over the three genetic models. This result suggests that even if the multiple quantitative traits are not distributed as multivariate normal distribution, the robust test MAX_m may still be valid for testing association.

Power Performance of the Multivariate Association Tests over the Extent of Degree of Dominance

In order to address the disparity of power performances of the multivariate association tests with respect to the degree of dominance t, in this subsection we assess the power performances of the whole extent [0, 1] of t with a step size 0.1 in the situations of $p = 0.2$ and $p = 0.5$ as the two quantitative traits were generated from the bivariate normal distribution with correlation coefficient 0.3. For each situation, the displacement effects d_1 and d_2 were set to allow the heritabilities of the two quantitative traits around 2% and 3%, respectively. These simulation results are depicted in Figures 1 and S1. From the curve patterns in Figure 1 for $p = 0.2$, we see that the farther the degree of dominance t deviates from that of the true genetic model, the more power loss appears on the optimal multivariate score test X^2_{REC} or X^2_{DOM}. It is noteworthy that the testing powers of X^2_{ADD}, FBAT-GEE and FBAT-Tau have a similar curve pattern over the whole extent of t, which indicates that the three tests may lose reliable capability to detect the authentic association between a putative QTL gene and the two quantitative traits when the value of t approaches 0. In contrast, the robust multivariate association test MAX_m reveals relatively stable power performances over the whole extent of t. The curve patterns in Figure S1 for $p = 0.5$ are similar to those in Figure 1 and, in particular, they exhibit a symmetric pattern over the whole extent of degree of dominance.

An Application to GAW 14 COGA Data

The COGA, which provided one of the data sets made available by the Genetic Analysis Workshop 14, is a nine-site national collaborative family study, which aims at mapping the genes to characterize the susceptibility of alcohol dependence and related phenotypes (Begleiter et al., 1995; Edenberg, 2002; Edenberg et al., 2005). The COGA data consist of 143 pedigrees with a total of 1614 individuals. The genetic data collected for each individual include multiple alcohol-related phenotypes and 328 highly polymorphic microsatellite markers for a 10 cM genome map. Previous results suggested that electrophysiological traits are highly heritable phenotypes associated with the risk of alcoholism (Porjesz et al., 2002) and reported that linkage signals were detected for alcohol dependence on Chromosome 4 (Long et al., 1998; Corbett et al., 2005; Prescott et al., 2006). Here, we chose two electrophysiological quantitative traits; ttth1 (the data from the visual oddball experiment, measured from far frontal left side channel) and ecb21 (the data from the eyes closed resting electroencephalography experiment) for analysis. To analyze the COGA data with the proposed method, the 143 pedigrees were first divided into 1109 triad families. A family of these restructured triad families would be actually included in the analysis if data from at least one of the two traits, ttth1 and ecb21, were available in the offspring of the family. Overall, of the 1109 triad families, 871 families conformed to the criterion and were drawn for analysis. For a family of a missing trait, that trait was imputed by the corresponding mean trait value of the offspring of the 871 target families. In summary, associations between the two quantitative traits, ttth1 and ecb21, and the 17 selected microsatellite markers on Chromosome 4, were evaluated using 871 informative triad families. To avoid being overly conservative when Bonferroni correction is applied to adjust for multiple testing for the following robust association analyses, the pairwise correlations among the 17 markers were examined. The 136 correlation coefficients range from 0.0022 to 0.1802. Because tests for significance show that these correlations are either nonsignificant or slightly significant, all 17 markers were included in the analysis. Moreover, because our proposed robust multivariate association test was constructed under the assumption of multivariate normal distribution given each QTL genotype, here, as was that of Chen et al. (2005), we also used log-transformation values for ttth1 and ecb21 to cope with the skewness of the two adopted electrophysiological traits. The coefficients of correlation between the two traits are calculated as 0.1086 ($P = 0.0013$) and 0.1394 ($P = 0.00004$) for the raw and log-transformed data, respectively. Basically, the two traits can be viewed as weakly correlated. To demonstrate the applicability of our proposed robust multivariate association test MAX_m, we compared it with the FBAT-GEE (Lange et al., 2003) and FBAT-Tau (Zhang et al., 2010) methods and with the univariate maximin efficiency robust test (MERT) proposed by Wang & Tai (2009). In the calculation of the P-values of MAX_m, $B = 10{,}000$ null samples were evaluated for the resampling-based procedure.

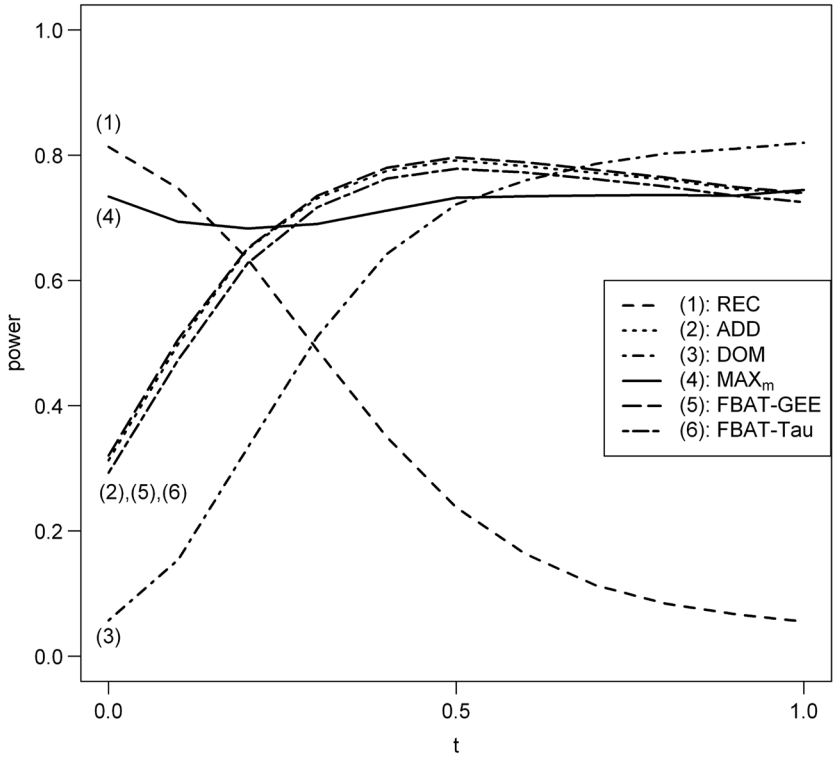

Figure 1 Power performances of the six multivariate association tests with respect to the degree of dominance t under overall genetic models where the heritabilities of the two quantitative traits are controlled around 2% and 3%, respectively. Allele frequency $p = 0.5$ and correlation coefficient $\rho = 0.3$.

Figure 2 displays the results of the MAX_m, FBAT-GEE and FBAT-Tau tests for association between the two log-transformed quantitative traits ttth1 and ecb21 and the 17 markers on Chromosome 4. Figure 2 shows that a higher peak is observed at the location of marker D4S1629. The P-values of the MAX_m, FBAT-GEE and FBAT-Tau on marker D4S1629 are calculated as 0.0019, 0.0179, and 0.0280, respectively. After Bonferroni adjustment for multiple comparisons among the 17 markers, only the robust multivariate association test MAX_m reached statistical significance at a level of $\alpha_{Bon} = 0.05/17 = 0.0029$. It can be noted that the optimal multivariate score test for dominant model X^2_{DOM} also showed a strong association with a P-value of 0.00067. These results indicate that the mode of inheritance of the candidate locus D4S1629 could be dominant. This explains why the two additive genetic model-based methods, FBAT-GEE and FBAT-Tau, do not have enough power to detect association in the case studied here. Figure S2 shows the results of robust univariate and multivariate association tests between the two log-transformed quantitative traits ttth1 and ecb21 and the 17 markers on Chromosome 4. The P-values of MERT on marker D4S1629 corresponding to the quantitative traits ttth1 and ecb21 are 0.0424 and 0.0597, respectively. After Bonferroni correction, the two values cannot exceed the adjusted level of $\alpha_{Bon} = 0.05/(2 \times 17) = 0.0015$. This result indicates that the proposed MAX_m test can indeed extract exclusive association information from both traits and is thus more powerful than either univariate robust association test in the detection of a putative locus for the traits studied.

Discussion

When studying complex diseases, researchers often collect multiple quantitative traits to shed light on the genetic etiology of the diseases (e.g., the metabolic disease risk study by Small et al., 2011 and the psychiatric disorder study by Edwards et al., 2012). In practical studies, one can analyze the multiple traits data by performing multiple one-trait analysis procedures to reach an overall conclusion. However, such a procedure would encounter the problem of multiple comparisons among multiple quantitative traits. To circumvent this

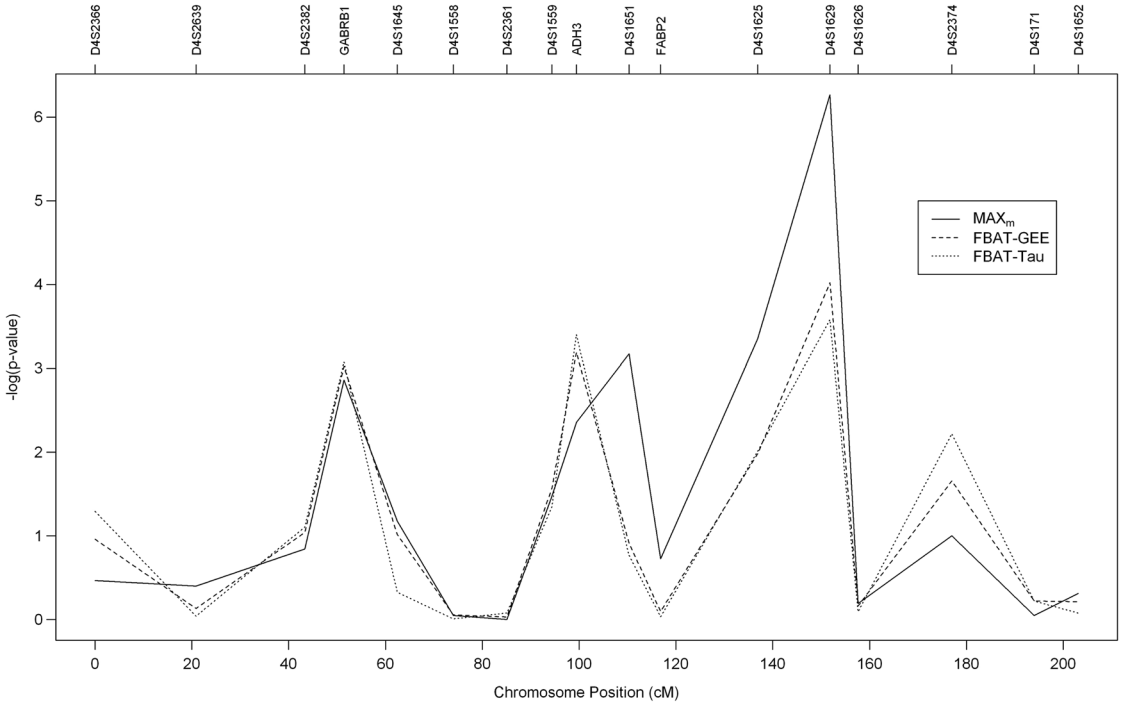

Figure 2 Results of multivariate association analysis between the two log-transformed traits ttth1 and ecb21 and the 17 markers on Chromosome 4 using the robust test MAX_m (solid line), FBAT-GEE (dashed line), and FBAT-Tau (dotted line) methods. The horizontal axis represents the physical distance of the 17 markers on Chromosome 4, and the vertical axis represents the negative log-transformed *P*-value of association analysis.

problem, the multivariate analysis procedures FBAT-GEE and FBAT-Tau were proposed (Lange et al., 2003; Zhang et al., 2010). The joint analysis of multiple traits would not only avoid the problem of multiple comparisons but would also enhance the power of genetic association analysis. Since the two tests were developed based on the allele-counting method for modeling the genotypic effect, they can actually be viewed as derived under the additive model (Li et al., 2008a). The similar power performances of X^2_{ADD}, FBAT-GEE and FBAT-Tau, as shown by our simulation results in Figures 1 and S1, confirm this point, due to the fact that the three tests are all constructed under the assumption of the additive model. Our simulation results also show that the proposed robust multivariate association test MAX_m has relatively stable power performance over all plausible genetic models for testing the association between a QTL gene and multiple quantitative traits. Comparisons among the MAX_m, X^2_{ADD}, FBAT-GEE, and FBAT-Tau indicate that MAX_m has comparable power to the additive model-based tests X^2_{ADD}, FBAT-GEE, and FBAT-Tau when the underlying genetic model is additive. According to these results, it should be highlighted that since FBAT-GEE and FBAT-Tau can also be computed under the recessive and dominant models, the MAX-type statistics can be constructed for these tests as well. Given that the comparison of FBAT-GEE and FBAT-Tau shows no power advantage over the proposed test here under the additive model, it would be plausible to assume that all three approaches should also have similar power when a MAX-type statistic approach is applied to FBAT-GEE and FBAT-Tau under all three genetic models. Because the merit of MAX_m is its power performance in robustness, especially when the underlying genetic model is moving toward the recessive or dominant model, and, additionally, because previous and current results show that the association tests based on additive model assumption are still valid, to some extent, under the models other than the additive, it is suggested that in real studies one can conduct those existing multivariate association tests at first, and then consider the MAX-type statistics for verification of association results when the values of those additive model-based test statistics are relatively low.

The analysis results of the COGA data show that the MAX_m is capable of detecting the association between the traits ttth1 and ecb21 and the marker D4S1629, but the robust univariate test MERT is not. This result indicates that analysis of multiple traits data using a robust univariate test like MERT with Bonferroni adjustment may result in a loss of power in

the detection of association. In other words, we can expect that the robust multivariate association tests should be more powerful than the robust univariate association tests when dealing with multiple quantitative traits. Finally, although the method proposed here is established under the assumption of multivariate normal distribution, the simulation results indicate that it is still a valid test as the underlying distribution deviated from the multivariate normal distribution. In addition, extending the method to adjust for important covariates should be addressed in future studies.

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Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Skewness coefficients of the bivariate gamma distribution under the simulation settings defined in Table S2 (b). Skew1 and Skew2 are the skewness for the traits $y^{(1)}$ and $y^{(2)}$ given each specific QTL genotype.

Table S2 Simulation settings of the displacement effects d_1 and d_2 and their corresponding heritabilities H_1^2 and H_2^2 under the three allele A frequencies and different genetic models for the two correlated quantitative traits with correlation coefficient $\rho = 0.3$.

Table S3 Comparisons of empirical type I error rates under the null hypothesis and power under the three true models among the three optimal multivariate score tests (X^2_{REC}, X^2_{ADD}, and X^2_{DOM}), the three robust multivariate association tests (MAX_m, MAX_{RA}, and MAX_{AD}) and the two existing multivariate methods (FBAT-GEE and FBAT-Tau) under the scenario that the two quantitative traits are distributed as bivariate gamma with correlation coefficient 0.3. Allele frequency of the QTL is 0.1, 0.2, or 0.5, and sample size is 500.

Table S4 The transmission probabilities $p_{j|i}$, the conditional expectations of the first derivative D_ℓ, and the conditional covariances between any two first derivatives D_ℓ and $D_{\ell'}$ under the three genetic models for each parent–offspring combination (i, j) defined in Table 1.

Figure S1 Power performances of the six multivariate association tests with respect to the degree of dominance t under overall genetic models where the heritabilities of the two quantitative traits are controlled around 2% and 3%, respectively; allele frequency $p = 0.5$ and correlation coefficient $\rho = 0.3$.

Figure S2 Results of association analysis between the two log-transformed traits ttth1 and ecb21 and the 17 markers on Chromosome 4 using the robust univariate and multivariate tests.

Appendix: Derivation of the optimal multivariate score test statistics X^2_{REC}, X^2_{ADD}, and X^2_{DOM}.

Software: An R-script to implement the proposed three specific genetic model-based multivariate score tests and the robust multivariate association test, as well as a brief manual are available in Supporting Information.

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