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A genome-wide association study of multiple longitudinal traits with related subjects

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Abstract

Pleiotropy is a phenomenon that a single gene inflicts multiple correlated phenotypic effects, often characterized as traits, involving multiple biological systems. We propose a two-stage method to identify pleiotropic effects on multiple longitudinal traits from a family-based data set. The first stage analyzes each longitudinal trait via a three-level mixed-effects model. Random effects at the subject-level and at the family-level measure the subject-specific genetic effects and between-subjects intraclass correlations within families, respectively. The second stage performs a simultaneous association test between a single nucleotide polymorphism and all subject-specific effects for multiple longitudinal traits. This is performed using a quasi-likelihood scoring method in which the correlation structure among related subjects is adjusted. Two simulation studies for the proposed method are undertaken to assess both the type I error control and the power. Furthermore, we demonstrate the utility of the two-stage method in identifying pleiotropic genes or loci by analyzing the Genetic Analysis Workshop 16 Problem 2 cohort data drawn from the Framingham Heart Study and illustrate an example of the kind of complexity in data that can be handled by the proposed approach. We establish that our two-stage method can identify pleiotropic effects whilst accommodating varying data types in the model.

Keywords

genetic association study; longitudinal data; mixed effects model; multiple traits; pleiotropy; quasi-likelihood; single nucleotide polymorphisms

1. Introduction

Genetic aetiology of complex diseases, such as type 2 diabetes and cardiovascular disease (CVD), has identified genetic elements as common, contributing factors to these diseases. However, identification of specific genes that predispose humans to these complex diseases has been difficult (Newman et al., 2011). It is suspected that these diseases have complex combinations of genetic components and non-genetic elements that contribute to their occurrences. In genome-wide association studies (GWAS), hundreds of thousands of genetic variants are tested for their individual association with a phenotypic trait of interest. GWAS

are considered to be a practical approach in screening the entire human genome for disease-associated loci via common genetic variants such as single nucleotide polymorphisms (SNPs) (O'Reilly et al., 2012; Solovieff et al., 2013). GWAS has become practical as the cost of acquiring a dense panel of SNPs has become more affordable. Complex phenotypic traits may be governed by multiple genes and environmental factors, and are subjective and *ad hoc* in nature. On the contrary, in general, genotypes are definitive entities. Therefore, it is the core objective of GWAS to characterize those phenotypic traits that are well-defined in their biological associations with complex diseases by genotypes.

Pleiotropy is a genetic phenomenon in which a single gene or genetic variant imposes two or more correlated phenotypic effects, often characterized as traits, involving two or more biological systems. A study of pleiotropic genes or loci may provide new knowledge about the evolution of genes and gene families as they relate to the aetiology of complex diseases (Hodgkin, 1998). The recent emergence of multiple-trait analysis in GWAS was not unforeseen, as clinical and epidemiological studies in humans capture multiple phenotype information (Shriner, 2012). For example, the Framingham Heart Study (FHS) includes multiple phenotypic measures, such as measurements of systolic blood pressure (SBP), total and high-density lipoprotein (HDL) cholesterol, and fasting glucose, to identify common characteristics that contribute to CVD; note that the aforementioned quantitative traits are now known to be some of the major risk factors of CVD. Shriner (2012) states that the statistical advantages of joint analysis of correlated traits include increased power to detect loci and increased precision of parameter estimation. Furthermore, performing joint analysis of correlated traits provides a means to (1) address the issue of varying types of pleiotropy and (2) investigate endophenotypes of complex traits, and thereby to better our understanding of the aetiology of complex diseases. A simple, traditional method for investigating pleiotropy involves multiple univariate analyses, in which a hypothesis test for an association between a genetic variant (e.g., SNP genotypes as the covariate) and a single trait (as the response variable) is performed for all complex traits in question over hundreds of thousands of genetic variants. This requires a subsequent step to determine whether or not the genetic variant is significantly associated with more than one trait. Inflation of family-wise error rate (FWER) is of concern when performing multiple hypothesis tests, especially with an increasing number of phenotypic traits (Feng, 2014; Wang et al., 2014). There are other methods proposed to analyze multiple correlated traits such the methods based on the multivariate linear mixed models and the principal-component analysis (Zhou & Stephens, 2014; Stephens, 2013; Aschard et al., 2014).

Longitudinal studies provide well-documented advantages over cross-sectional studies, but longitudinal studies have their challenges. (Hedeker & Gibbons, 2006). To gain power in detecting strongly associated SNPs or genes, we attempt to take full advantage of utilizing these available longitudinal data to lay the foundations for more reliable causal inference. One particular advantage of longitudinal studies is the ability to model a dynamical system within subjects and state statistical propositions about the dynamical system through statistical inferences. Furthermore, the inclusion of repeated measurements of time-varying covariates in the model permits much stronger statistical inferences about this dynamical system. However, the presence of missing data and the dependency in data impart significant complexity to the statistical modelling of longitudinal data (Hedeker & Gibbons, 2006). We

overcome some of these challenges and gain positive features from conducting a longitudinal study via generalized linear mixed models (GLMMs). Application of GLMMs in longitudinal studies relaxes restrictive assumptions about the variance-covariance structure of the repeated measurements and missing data across time. GLMMs are quite robust to missing data and repeated measurements taken at unequal time points, thereby allowing analysis of unbalanced longitudinal data according to large sample theory (Hedeker & Gibbons, 2006). Furthermore, GLMMs conveniently accommodate both time-invariant and time-varying covariates. A particular feature of longitudinal studies we aim to exploit is their multi-level data structures. The use of all available data from each subject in a longitudinal study via GLMMs enables us to predict both subject-specific and family-specific effects, leading to increased statistical power and decreased bias due to attrition (Hedeker & Gibbons, 2006). The methods for genetic association analysis with the longitudinal traits can also be found in Sikorska et al. (2013); Furlotte et al. (2012). However, Sikorska et al. (2013) state that fitting linear mixed models to the longitudinal data can be computationally demanding and propose a computationally feasible solution for investigating the association between the given SNP and the longitudinal trait of interest. Their method is an approximate method based a conditional two-step approach.

Family-based genome-wide SNP data with rare genetic variants and a complex pedigree structure pose problems of high dimensionality. While performing population-based GWAS is a simpler approach, it is susceptible to population stratification (Feng et al., 2011). Hence, effectively incorporating family-based designs in GWAS can provide robustness to the effect of population stratification in allele frequencies (Naylor et al., 2010). Newman et al. (2011) emphasize that failure to account for pedigree relationships affects statistical tests of association. In GWAS of multiplex families, affected subjects (e.g., subjects with type 2 diabetes) with affected, biologically related subjects have a higher expected frequency of the allele that predisposes them to exhibit closely associated genetic conditions than do affected subjects with no affected, related subjects. As a result, the power to detect genetic association is expected to increase when affected subjects with affected, related subjects are included in the study. When related subjects are used in association studies, it is critical to account for the fact that subjects who are biologically related have correlated genotypes (Thornton & McPeck, 2007). The generalized quasi-likelihood scoring method (GQLSM) is an extension of the generalized linear model framework, proposed by Feng (2014), that was designed to accommodate variables other than binary type. Furthermore, its capacity to integrate the correlation structure among related individuals was inherited from the derivatives of the quasi-likelihood scoring framework introduced by Bourgain et al. (2003) and Thornton & McPeck (2007). In studies of complex diseases, it is inevitable that different types of data are used to express the phenotypic traits and that multiple data types (e.g., binary, ordinal, count, quantitative) are collected. Feng (2014) emphasizes that having a model that can not only accommodate a variety of data types but that can simultaneously analyze these varying data types is desired and can provide a powerful tool in the field of statistical genetics. Here, we also address the confounding effects caused by the population stratification by proposing a robust method to the effects imposed by population structure, e.g., the confounding effect of ethnicity is well recognized as the effect of population heterogeneity in genetics literature (Feng et al., 2011).

We extend the two-step strategy introduced by Wang et al. (2014) and design an alternative statistical method to accommodate cases when the assumption of independent subjects is violated. We propose a two-stage method to identify pleiotropy on multiple longitudinal traits from family-based data. First, we analyze each longitudinal trait via a three-level mixed-effect model in which the repeated measurements are nested within subjects and the subjects are nested within families. Random effects predicted at the subject-level and the family-level, via GLMMs, represent the subject-specific genetic effects and between-subject intraclass correlations within families, respectively. Second, we perform a simultaneous association test between an SNP and all subject-specific effects for multiple longitudinal traits. The genetic association test is based on the GQLSM in which the correlation structure among related subjects is adjusted.

Our manuscript is organized as follows. In Section 2, we provide an overview of the proposed statistical method. The details about the simulation studies and their results are shared in Section 3. We applied the proposed method to analyze the Genetic Analysis Workshop 16 (GAW16) Problem 2 data drawn from the FHS. Section 4 provides descriptions of the original GAW16 Problem 2 data set, pre-processing steps taken for our analysis, and key findings from the analysis of GAW16 Problem 2 data. Discussion about the results and recommendations for future research are described in Section 5.

2. Statistical Methods

2.1. Generalized Linear Mixed Models for Longitudinal Traits

We model longitudinal data via a generalized linear mixed model (GLMM). In particular, each longitudinal phenotypic trait is modelled using a three-level mixed-effect model. Here, random effects at the subject-level and at the family-level measure the subject-specific genetic effects and between-subjects intraclass correlations within families, respectively. The random effects allow the correlation between the repeated measurements to be incorporated into the estimates of parameters, standard errors, and tests of hypotheses. We can conceptualize the random effects at the subject-level as representing subject-specific differences in the propensity to response over time, conditional on their values of fixed effects included in the model (Hedeker & Gibbons, 2006).

Suppose we have a sample consisting of F independent families in an outbred population. Among n subjects, let n_i be the number of subjects that are from the i^{th} family. Then, we have the sample size of $n = n_1 + \dots + n_i + \dots + n_F$. Let $\mathbf{X}_{ijk} = (X_{ijk1}, \dots, X_{ijkb}, \dots, X_{ijkT_{ijk}})'$ be the vector of T_{ijk} measurements of the k^{th} trait of the j^{th} subject from the i^{th} family. A general form of a generalized linear mixed model (GLMM) is given by

$$g_k(\mu_{ijk}) = \eta_{ijk} = \mathbf{Z}'_{ijk} \mathbf{a}_k + \Gamma_{ik} + \gamma_{ijk}, \quad (1)$$

where $g_k(\cdot)$ is the link function for the k^{th} trait; μ_{ijk} , η_{ijk} , and $\mathbf{Z}_{ijk}$ are the conditional mean of X_{ijk} given $\mathbf{Z}_{ijk}$, linear predictor, and vector of covariates associated with the k^{th} trait, respectively, for the j^{th} subject from the i^{th} family at time t ; $\mathbf{a}_k$ is a vector of fixed effects for covariates $\mathbf{Z}_{ijk}$; Γ_{ik} is the i^{th} family-specific effect (random effect) on the k^{th} trait; γ_{ijk} is the

j^{th} subject-specific effect (random effect) on the k^{th} trait. Note that $\mathbf{Z}_{ijk t}$ can take on both time-varying and time-invariant covariates. For example, body-mass index (BMI) is a time-varying covariate and a well-known risk factor for CVD. A covariate such as a subject's sex is time-invariant and is assumed to be constant over the course of a longitudinal study. We provide the flexibility to choose different sets of covariates to be included in the GLMMs for different longitudinal traits as denoted by the subscript k in $\mathbf{Z}_{ijk t}$, where $k = 1, 2, \dots, K$. Moreover, the number of repeated measurements can vary from subject to subject as denoted by the subscripts j and t , where $j = 1, 2, \dots, n_i$ and $t = 1, 2, \dots, T_{ijk}$. The family-specific effect Γ_{ik} can be defined as the effect of shared environmental factors, not accounted for by the confounding covariates, for the i^{th} family on their repeated measurements on the k^{th} trait. Similarly, we define the subject-specific effect γ_{ijk} as the influence of the j^{th} subject on his or her repeated measurements on the k^{th} trait, which captures the unobservable effects of major genes and polygenes. For k^{th} trait, for simplicity, we assume that the Γ_{ik} 's follow a normal distribution with a mean of 0 and a k^{th} trait-specific variance $\sigma_{\Gamma_k}^2$ and the γ_{ijk} 's follow a normal distribution with a mean of 0 and a k^{th} trait-specific variance $\sigma_{\gamma_k}^2$. Note that it is not necessary for the γ_{ijk} and Γ_{ik} to follow a normal distribution.

For a continuous k^{th} trait, a GLMM becomes a linear mixed-effect model such that

$X_{ijk t} = \mathbf{Z}_{ijk t}' \mathbf{a}_k + \Gamma_{ik} + \gamma_{ijk} + \varepsilon_{ijk t}$, where a random error $\varepsilon_{ijk t}$ is assumed to follow a normal distribution with a mean of 0 and a k^{th} trait-specific variance $\sigma_{\varepsilon_k}^2$. In this example, $g_k(\cdot)$ is an identity link such that $g_k(\mu_{ijk t}) = \mu_{ijk t}$. We fit the GLMM, using the 'lme4' package in R, to obtain a predicted j^{th} subject-specific effect from the i^{th} family on the k^{th} trait, γ_{ijk} (Bates et al., 2015a,b; Bates, 2014a,b). We denote these predicted subject-specific effects for the k^{th} trait as a vector $\hat{\boldsymbol{\gamma}}_k = (\hat{\gamma}_{11k}, \dots, \hat{\gamma}_{1n_1k}, \dots, \hat{\gamma}_{F1k}, \dots, \hat{\gamma}_{Fn_Fk})$. Then, we set the predicted subject-specific effects $\hat{\boldsymbol{\gamma}}_k$ on the k^{th} trait as the covariates in the second stage to perform a simultaneous association test between an SNP and all subject-specific effects for the k^{th} longitudinal trait. Furthermore, it is worth noting here that the fixed effects associated with confounding covariates are estimated using the GLMMs. As you will see in Section 3, if we have a longitudinal, binary trait $X_{ij\beta t}$ (e.g., hypertensive status), we can interpret the association between the subject-specific effect γ_{ijk} and the hypertensive status accordingly. We may state that the subject-specific effect γ_{ijk} is the underlying genetic risk factors for the j^{th} subject, from the i^{th} family, that affects the log-odds of experiencing hypertension. Recall that this is the case because, for a binary trait, a logistic link can be used with $g_k(\mu_{ijk t}) = \log[\mu_{ijk t}/(1 - \mu_{ijk t})]$ (Wang et al., 2014).

2.2. Genetic Association Study with Multiple Longitudinal Traits

From Section 2.1, we have acquired the predicted subject-specific effects on K traits, $\hat{\gamma}_1, \hat{\gamma}_2, \dots, \hat{\gamma}_K$, for a sample of n subjects from F independent families. For a given SNP, let $\mathbf{Y}_i = (Y_{i1}, \dots, Y_{in_i})'$ represent the observed genotypes of subjects from the i^{th} family with Y_{ij} defined as the proportion of allele 1 in the observed genotype of the j^{th} subject from the i^{th} family, i.e.,

$$Y_{ij} = \frac{1}{2} \times (\text{the number of allele 1 observed in the } j^{\text{th}} \text{ subject from the } i^{\text{th}} \text{ family}),$$

where $Y_{ij} = 0, \frac{1}{2}$, or 1 for all $i = 1, 2, \dots, F$, and $j = 1, 2, \dots, n_i$. Under the Hardy-Weinberg equilibrium, $2Y_{ij}$ follows Binomial(2, π_{ij}), where π_{ij} is the expected frequency of allele 1 for the given SNP for the j^{th} subject in the i^{th} family. Then, we arrange the response vector such that $\mathbf{Y} = (\mathbf{Y}'_1, \dots, \mathbf{Y}'_F)'$, and the overall design matrix is of the form

$\boldsymbol{\gamma} = (\boldsymbol{\gamma}'_1, \boldsymbol{\gamma}'_2, \dots, \boldsymbol{\gamma}'_i, \dots, \boldsymbol{\gamma}'_F)'$, where $\boldsymbol{\gamma}_i$ is an n_i by $(K+1)$ design matrix with its first column consisting of 1's. In $\boldsymbol{\gamma}_i$, the $(k+1)^{th}$ column represents the subject-specific effects corresponding to the k^{th} longitudinal trait for all subjects in the i^{th} family. The j^{th} row of the design matrix contains a 1 for the intercept and the K subject-specific effects for the j^{th} subject from the i^{th} family.

Feng (2014) proposes a logistic regression model to link the expected allele frequency

$\boldsymbol{\pi} = (\pi'_1, \dots, \pi'_F)'$ of allele 1 with multiple traits. Here, we treat K subject-specific effects as K phenotypic traits, so

$$\pi_{ij} = E(Y_{ij} | \boldsymbol{\gamma}_{ij}) = \frac{\exp(\boldsymbol{\gamma}'_{ij} \boldsymbol{\beta})}{1 + \exp(\boldsymbol{\gamma}'_{ij} \boldsymbol{\beta})} = \frac{\exp(\beta_0 + \beta_1 \gamma_{ij1} + \dots + \beta_K \gamma_{ijK})}{1 + \exp(\beta_0 + \beta_1 \gamma_{ij1} + \dots + \beta_K \gamma_{ijK})}. \quad (2)$$

If an SNP is associated with a longitudinal trait, it should be associated with its corresponding subject-specific effect, which includes the contribution of the SNP to the variation of the trait. Otherwise, the SNP would not be associated with the subject-specific effect if it is not associated with the longitudinal trait, and so the corresponding coefficient, say β_k , should be 0. Then, an overall test of the association between an SNP and a set of longitudinal traits can be formulated as

$$\begin{aligned} H_0: \beta_1 = \beta_2 = \dots = \beta_k = \dots = \beta_K = 0 \text{ against} \\ H_a: \text{At least one } \beta_k \neq 0, k=1, 2, \dots, K. \end{aligned}$$

Here, the null hypothesis corresponds to the situation when the SNP is not associated with any one of the K longitudinal traits. Moreover, a logistic regression model provides the natural constraint that $\pi_{ij} \in (0, 1)$ for all i and j . Under the null hypothesis, the mean of

response Y_{ij} given subject-specific effects on K traits $\boldsymbol{\gamma}_{ij}$ simplifies to $\pi_{ij} = \pi = \frac{\exp(\beta_0)}{1 + \exp(\beta_0)}$. Thus, the mean response vector becomes a constant vector in the form of $\boldsymbol{\pi} = E(\mathbf{Y} | \boldsymbol{\gamma}) = E(\mathbf{Y}) = \boldsymbol{\pi} \mathbf{1}$. Under the null hypothesis, the overall covariance matrix of $\mathbf{Y}$ has the form

$\sum_0 = \frac{1}{2} \pi (1 - \pi) \boldsymbol{\rho}$, where $\boldsymbol{\rho}$ is the overall correlation matrix. The $\boldsymbol{\rho}$ is a block diagonal matrix, where the diagonal elements are the $\boldsymbol{\rho}_i$'s for $i = 1, \dots, F$. Each $\boldsymbol{\rho}_i$ represents the correlation among subjects from the i^{th} family and zero matrices in the off-diagonal blocks represent the correlations among independent families. Within a family, the correlation matrix $\boldsymbol{\rho}_i$ can be calculated by the kinship and inbreeding coefficients based on the known relationships. For example, the correlation matrix of $\mathbf{Y}_i$ is given by

$$\rho_i = \begin{pmatrix} 1+\phi_1 & 2\phi_{12} & \cdots & 2\phi_{1n_i} \\ 2\phi_{21} & 1+\phi_2 & \cdots & 2\phi_{2n_i} \\ \vdots & \vdots & \ddots & \vdots \\ 2\phi_{n_i1} & 2\phi_{n_i2} & \cdots & 1+\phi_{n_i} \end{pmatrix},$$

where ϕ_j is the inbreeding coefficient of the j^{th} subject from the i^{th} family and ϕ_{ij} is the kinship coefficient between the j^{th} subject and the i^{th} subject in the i^{th} family. With an outbred population, $\phi_j = 0$ for all j . Note that the requirement of known relationships can be relaxed if genome-wide genetic data are available from which the relationships can be inferred. The quasi-likelihood score functions are in a $(K+1)$ -vector that has the form

$$U(\boldsymbol{\beta}) = (U_{\beta_0}(\boldsymbol{\beta}), U_{\beta_1}(\boldsymbol{\beta}), \dots, U_{\beta_K}(\boldsymbol{\beta})) = \mathbf{D}' \boldsymbol{\Sigma}^{-1} (\mathbf{Y} - \boldsymbol{\pi}), \quad (3)$$

where $\mathbf{D}$ is an $n \times (K+1)$ derivative matrix of the form

$\mathbf{D} = \frac{\partial \boldsymbol{\pi}}{\partial \boldsymbol{\beta}} = \left(\frac{\partial \boldsymbol{\pi}}{\partial \beta_0}, \frac{\partial \boldsymbol{\pi}}{\partial \beta_1}, \dots, \frac{\partial \boldsymbol{\pi}}{\partial \beta_K} \right)$, and $\boldsymbol{\Sigma}$ is the covariance matrix of $\mathbf{Y}$. Under the null hypothesis that $\boldsymbol{\beta}_{-0} = (\beta_1, \beta_2, \dots, \beta_K)' = 0$, the mean response vector $\boldsymbol{\pi} = \pi \mathbf{1}$, the covariance matrix $\boldsymbol{\Sigma} = \Sigma_0$, $\mathbf{D} = \pi(1 - \pi)\boldsymbol{\gamma}$, and $U(\boldsymbol{\beta}) = 2\boldsymbol{\gamma}'\boldsymbol{\rho}^{-1}(\mathbf{Y} - \pi\mathbf{1})$. Under the null hypothesis that $\boldsymbol{\beta}_{-0} = 0$, the estimate of π given by $\hat{\pi} = (\mathbf{1}'\boldsymbol{\rho}^{-1}\mathbf{1})^{-1}\mathbf{1}'\boldsymbol{\rho}^{-1}\mathbf{Y}$ or can be written as

$\hat{\pi} = \left(\sum_{i=1}^F \mathbf{1}_i' \boldsymbol{\rho}_i^{-1} \mathbf{1}_i \right)^{-1} \left(\sum_{i=1}^F \mathbf{1}_i' \boldsymbol{\rho}_i^{-1} \mathbf{Y}_i \right)$, where $\mathbf{1}_i$ is the n_i -vector of 1's. According to Cox & Hinkley (1974) and Heyde (1997), the quasi-likelihood score statistic is given by

$$W = U_{\boldsymbol{\beta}_{-0}}(\hat{\boldsymbol{\beta}}_0, 0)' \text{cov}_0^{-1}(U_{\boldsymbol{\beta}_{-0}}(\hat{\boldsymbol{\beta}}_0, 0)) U_{\boldsymbol{\beta}_{-0}}(\hat{\boldsymbol{\beta}}_0, 0), \quad (4)$$

where $U_{\boldsymbol{\beta}_{-0}}(\hat{\boldsymbol{\beta}}_0, 0)$ is a vector of score functions given by Equation 3 in which the score function for β_0 is omitted and $\text{cov}_0^{-1}(U_{\boldsymbol{\beta}_{-0}}(\hat{\boldsymbol{\beta}}_0, 0))$ is a $K \times K$ matrix where the first row and the first column of the inverse of the information matrix $\mathbf{I}(\boldsymbol{\beta})$ are omitted; these are computed under the null hypothesis that $\boldsymbol{\beta}_{-0} = 0$. From Feng (2014), the W statistic can be derived explicitly and is given as

$$W = \frac{2}{\hat{\pi}(1 - \hat{\pi})} (\mathbf{Y} - \hat{\pi}\mathbf{1})' \boldsymbol{\rho}^{-1} \boldsymbol{\gamma}_{-1} [(\boldsymbol{\gamma}' \boldsymbol{\rho}^{-1} \boldsymbol{\gamma})^{-1}]_{-1, -1} \boldsymbol{\gamma}_{-1}' \boldsymbol{\rho}^{-1} (\mathbf{Y} - \hat{\pi}\mathbf{1}), \quad (5)$$

or in an alternative form of

$$W = \frac{2}{\hat{\pi}(1 - \hat{\pi})} \left(\sum_{i=1}^F \boldsymbol{\gamma}_{i,-1}' \boldsymbol{\rho}_i^{-1} (\mathbf{Y}_i - \hat{\pi}\mathbf{1}_i) \right)' \left[\left(\sum_{i=1}^F \boldsymbol{\gamma}_{i,-1}' \boldsymbol{\rho}_i^{-1} \boldsymbol{\gamma}_i \right)^{-1} \right]_{-1, -1} \left(\sum_{i=1}^F \boldsymbol{\gamma}_{i,-1}' \boldsymbol{\rho}_i^{-1} (\mathbf{Y}_i - \hat{\pi}\mathbf{1}_i) \right).$$

Under the null hypothesis, the W statistic follows a χ^2 -distribution with the degrees of freedom determined by the rank of the matrix $\sum_{i=1}^F \gamma_i' \rho_i^{-1} \gamma_i$. Thus, if the K subject-specific effects being tested are linearly independent, then $W \sim \chi_K^2$ asymptotically. The latter form of the W statistic breaks down a large sample of size n into F independent families. As a result, it achieves computational feasibility by circumventing the manipulation of high dimensional matrices. As mentioned in Feng (2014), when a single k^{th} trait is tested, i.e., when a k^{th} trait is tested individually, the W statistic for testing the association between an SNP and the k^{th} trait can be rewritten as

$$W_k = \frac{2}{\hat{\pi}(1 - \hat{\pi})} (\mathbf{Y} - \hat{\pi} \mathbf{1})' \boldsymbol{\rho}^{-1} \boldsymbol{\gamma}_k [(\boldsymbol{\gamma}' \boldsymbol{\rho}^{-1} \boldsymbol{\gamma})^{-1}]_{-1, -1} \boldsymbol{\gamma}_k' \boldsymbol{\rho}^{-1} (\mathbf{Y} - \hat{\pi} \mathbf{1}), \quad (6)$$

where $\boldsymbol{\gamma} = (\mathbf{1}', \boldsymbol{\gamma}_k')'$ and $W_k \sim \chi_1^2$ asymptotically.

3. Simulation Studies

3.1. Simulation Models and Methods

To assess the performance of the proposed two-stage method, we conducted simulation studies evaluating the type I error rate and the power of the association tests. The assessment of power compares the power obtained by testing multiple traits simultaneously with the power achieved by testing each trait individually.

To generate a family data set, we grow a family starting from two unrelated subjects as a couple. Note that we define these unrelated subjects in a family whose parental information is unknown as founders. For each couple, the number of offspring is generated according to a Poisson distribution with a mean of 3. Each offspring is then assigned an unrelated subject as a spouse with a probability of 0.8 to form an offspring couple. Then, a grand-offspring of this offspring couple is generated from a Poisson distribution with a mean of 3. Note that the unrelated spouse is defined as a founder as well. We grow a family for up to three generations. It may be the case that a family stops growing before the completion of three generations by the process of natural degeneration. As a result, we generate families that are made up of two to 36 subjects with a mean size of about 9 subjects per family. The genealogy of each family remains for calculating the correlation matrix $\boldsymbol{\rho}$.

Two simulation studies with 1 000 simulation replicates per study are implemented, each with sample sizes of $n = 300, 500$, and 1000 . In both studies, we consider two quantitative traits X_1 and X_2 , and one binary trait X_3 . These traits can be affected by five causal SNPs, denoted by $G_1, G_2, \dots, G_5$, at different levels, i.e., each SNP affects at least one of the three traits. The effects of SNPs on the three traits are shown in Table 1. In Study 1, all five SNPs have genetic effects on all three traits at different levels. In Study 2, each of the five SNPs affects a different number of the three traits. For example, G_3 has a genetic effect on X_2 only, which is defined by setting the coefficients $b_{13} = 0$, $b_{23} = 0.16$, and $b_{33} = 0$ as shown in Table 1.

In practical situations, causal SNPs might not be genotyped. Instead, SNPs that are proximal to or in linkage disequilibrium (LD) with the causal SNPs are genotyped and available for the association analysis. To take this situation into account, we generate genotypes of both causal SNPs and SNPs that are in LD with the causal ones. We denote the SNPs that are in LD with the causal ones by $M_1, M_2, \dots, M_5$. For each subject, to generate the SNP genotypes, we generate haplotype pairs for each subject. A haplotype is referred as the combination of marker alleles on a single chromosome that were inherited as a unit from a single parent. We denote the haplotype for two SNPs G_r and M_r as $\mathbf{H}_r = (H_{G_r}, H_{M_r})$ for $r = 1, 2, \dots, 5$. H_{G_r} and H_{M_r} take a value of 1 for having allele 1 and 0 for not having allele 1. Given a family, haplotypes of founders are generated from a bivariate Bernoulli distribution

with a mean vector $\boldsymbol{\pi}_r = (\pi_{G_r}, \pi_{M_r})$ and a covariance matrix $\Sigma_r = \begin{pmatrix} \sigma_{G_r}^2 & \sigma_{G_r, M_r}^2 \\ \sigma_{G_r, M_r}^2 & \sigma_{M_r}^2 \end{pmatrix}$

where $\sigma_{G_r}^2 = \pi_{G_r}(1 - \pi_{G_r})$, $\sigma_{M_r}^2 = \pi_{M_r}(1 - \pi_{M_r})$, $\sigma_{G_r, M_r}^2 = \rho_r(\sigma_{G_r})(\sigma_{M_r})$, and the correlation for an r^{th} pair of SNPs, ρ_r is set at a fixed value between 0.7 and 0.9. By random mating, a pair of H_{G_r} and H_{M_r} are generated to make up the genotypes G_r and M_r for a founder. Haplotypes of non-founders (i.e., offspring) are generated according to the Mendelian Law of Segregation from each parent. Similarly, a pair of haplotypes $\mathbf{H}_r$ for an offspring makes up the genotypes of the two SNPs G_r and M_r for this offspring. Furthermore, for the assessment of type I error rate, ten independent SNPs that are not associated with any one of the three traits are generated. The results from the type I error rate assessments for these ten SNPs are accumulated in both studies, resulting in 10 000 simulation replicates per study.

Then, we generate two covariates Z_{ijt1} and Z_{ijt2} and a family-level random effect Γ_{ik} , where Z_{ijt1} is a binary covariate generated from $Bernoulli(0.3)$, Z_{ijt2} is a continuous covariate generated from $Gamma(\psi_g, \theta_g)$, and Γ_{ik} is a family-specific effect generated from $N(0, \sigma_{\Gamma_k}^2)$. Here, Z_{ijt1} is a time-varying, binary covariate that mimics the treatment status that may change over time. The second time-varying covariate Z_{ijt2} is generated to mimic the age of a subject that changes over time. With family data that include members over three generations, the parameters of $Gamma(\psi_g, \theta_g)$ are estimated empirically using the GAW16 Problem 2 data set in order to generate more realistic age data. For example, when $g = 1$, the empirical mean and variance of subject age in the grandparent generation are used to estimate ψ_1 and θ_1 , respectively. For each j^{th} subject in an i^{th} family, T_{ij} measurements of age are generated from $Gamma(\psi_1, \theta_1)$ and are sorted in an ascending order, where the j^{th} subject is a grandparent for $g = 1$. We repeat this process to generate the age for subjects in the second and third generations, i.e., for $g = 1$ and 2.

Given the generated covariates, genotypes of the causal SNPs, and the family-specific effect, we compute the linear predictor η_{ijkt} for each k^{th} longitudinal trait such that

$$\eta_{ijkt} = g_k(\mu_{ijkt}) = a_{k0} + a_{k1}Z_{ijt1} + a_{k2}Z_{ijt2} + \Gamma_{ik} + b_{k1}G_{ij1} + b_{k2}G_{ij2} + \dots + b_{k5}G_{ij5} \quad (7)$$

for $i = 1, \dots, F$, $j = 1, \dots, n_i$, $k = 1, \dots, K$, and $t = 1, \dots, T_{ij}$. Then, two quantitative traits X_{ij1t} and X_{ij2t} are generated from $N(\mu_{ij1t}, 1)$ and $N(\mu_{ij2t}, 1)$ with identity links $\eta_{ij1t} = \mu_{ij1t}$ and $\eta_{ij2t} = \mu_{ij2t}$, respectively. In addition, a binary trait X_{ij3t} is generated from $Bernoulli(\mu_{ij3t})$,

where $\mu_{ij\beta t} = \exp(\eta_{ij\beta d}) / (1 + \exp(\eta_{ij\beta d}))$. Table 1 summarizes the effects of SNPs on the longitudinal traits for Studies 1 and 2. For the fixed effects of covariates Z_{ij1t} and Z_{ij2t} , we set $\mathbf{a}_1 = (a_{10}, a_{11}, a_{12})' = (0, 0.3, 0.5)'$ for the first trait X_{ij1t} and $\mathbf{a}_2 = (a_{20}, a_{21}, a_{22})' = (0, 0.2, -0.3)'$ for the second trait X_{ij2t} . For the binary trait X_{ij3t} , we set $\mathbf{a}_3 = (a_{30}, a_{31}, a_{32})' = (-2.4, -1.6, 0.06)'$. We use the identical sets of fixed effects of covariates in both Study 1 and Study 2.

For each simulated data set, we first fit the GLMM, based on Equation 1, to obtain a predicted subject-specific effect γ_{ijk} , denoted as $\hat{\gamma}_{ijk}$, on each trait for the j^{th} subject in the i^{th} family. In the GLMM, both covariates Z_{ij1t} and Z_{ij2t} are included. Note that family-specific effects, Γ_{ik} 's, on each trait for F families are also predicted. However, the family-specific effects are not the focus of this manuscript and so the results related to the predicted family-specific effects, Γ_{ik} 's, are not shown. Again, the fitting of the GLMM is implemented using the 'lme4' package in R (Bates et al., 2015a,b; Bates, 2014a,b). The predicted subject-specific effects $\hat{\gamma}_{ijk}$'s are treated as phenotypes for the analysis in the second stage. For each SNP, we perform a simultaneous test on all three predicted subject-specific effects, $\hat{\gamma}_{ij1}$, $\hat{\gamma}_{ij2}$, and $\hat{\gamma}_{ij3}$, as in the overall hypothesis test. We compute the W statistic as given in Equation 5 and take the $(1 - \alpha_F)^{th}$ quantile of the χ_3^2 -distribution to be the rejection threshold. To perform the association test on each subject-specific effect, we compute the W_k statistic for $k = 1, 2, 3$, as given in Equation 6. The rejection threshold for the individual test is set at the $(1 - \alpha_F)^{th}$ quantile of the χ_1^2 -distribution, where α is obtained by solving $\alpha_F = 1 - (1 - \alpha)^3$ and α_F is the FWER that we try to control for multiple tests. We set $\alpha_F = 0.05, 0.01$, and 0.001 so that the corresponding α -levels for individual tests are set at $0.01667, 0.00333$, and 0.00033 , respectively.

3.2. Simulation Study Results

This section provides the results obtained from the simulation studies that evaluated the ability of the proposed two-stage method to control the type I error at the desired level of significance α_F , and to attain the statistical power for the given significance level over the multiple hypothesis testing procedure with the Bonferroni correction. Table 2 lists the mean and standard errors of the fixed effect coefficient estimates attained from fitting GLMMs for the three longitudinal traits in both studies. The fitting of the GLMMs under different combinations of settings generally yields estimates of the coefficients with small bias and standard errors.

3.2.1. Type I Error Rate Assessment—The empirical null rejection rates found in Table 3 are the summary of the accumulated null rejection rates from the ten SNPs that are not associated with any one of the traits in both Study 1 and Study 2. Under the null hypothesis, each SNP has 1000 simulation replicates. So we have 10 000 simulation replicates, in total, to assess the type 1 error rate. For the simultaneous tests, the empirical null rejection rates are very close to their corresponding nominal levels. For example, in Study 1, the empirical null rejection rates at $\alpha_F = 0.05$ are $0.0526, 0.0498$, and 0.0506 for samples sizes of $n = 300, 500$, and 1000 , respectively. The union of the longitudinal rejection rates reports the empirical FWER among the three traits. We observe that the empirical FWERs are very close to their corresponding nominal levels of α_F over all

different combinations of settings. For example, in Study 1, the empirical unions of null rejection rates over the three individual tests at $\alpha_F = 0.05$, or equivalently at $\alpha = 0.01667$, are 0.0516, 0.0478, and 0.0499 for sample sizes of $n = 300$, 500, and 1000, respectively.

3.2.2. Power Assessment—We compared the power achieved by the simultaneous association test with the power achieved by the individual tests. Tables 4–6 summarize the results for sample sizes of $n = 300$, 500, and 1 000 in Study 1. Similarly, Tables 7–9 summarize the results for sample sizes of $n = 300$, 500, and 1000 in Study 2. Note that the test with the higher power is indicated in boldface.

In Study 1, all causal SNPs, $G_1, \dots, G_5$, have influences on all three longitudinal traits. For each causal SNP, when performing the simultaneous association test on all traits, the power is consistently higher than the power obtained from the union of individual tests on each trait. When testing on SNPs, $M_1, \dots, M_5$, that are in LD with the causal SNPs, the power obtained from the simultaneous test is consistently higher than the power obtained from the union of individual tests on each trait. Moreover, as expected, the power is lower for testing on $M_1, \dots, M_5$ compared to the power obtained from testing on the causal SNPs $G_1, \dots, G_5$, correspondingly. The dilution of the power depends on the LD levels between the G_r and M_r for $r = 1, 2, \dots, 5$, and their allele frequencies.

In Study 2, we designed the causal SNPs to be associated with different numbers of traits: G_1 affects all three traits, G_2 and G_4 affect two traits, and G_3 and G_5 affect only one trait. The results are summarized in Tables 7–9. When the causal SNPs influence more than one trait, such as with G_1 , G_2 , and G_4 , the simultaneous association tests are consistently more powerful than the union of individual tests on each trait across different sample sizes. The power gain is more obvious if an SNP has effects on more traits. Note that G_2 is not associated with the second trait X_2 so the rejection rate should correspond to the type I error rate. So, these empirical type I error rates are highlighted in grey in Tables 7–9 to distinguish them from the empirical power. When the causal SNPs affect only one trait, such as with G_3 and G_5 , the power obtained from the simultaneous test is similar to the power obtained from the individual tests. Again, when SNPs $M_1, \dots, M_5$ that are in LD with the causal SNPs are tested, the power is generally lower. But, similar patterns in power to those obtained from the tests of the causal SNPs are observed.

4. Real Data Analysis

4.1. Data Description

We apply our proposed method to analyze the GAW16 Problem 2 data drawn from the FHS. The FHS is an ongoing, observational, prospective study for identifying CVD risk factors. The FHS is conducted under the supervision of the National Heart, Lung and Blood Institute and in collaboration with Boston University. The GAW16 Problem 2 data set is drawn from the FHS, and includes pedigree and phenotype data from three generations; Original Cohort, Offspring Cohort, and Third Generation Cohort were recruited from Framingham, Massachusetts, in 1948, 1971, and 2002, respectively, with four examinations of phenotypic traits collected repeatedly for the first two generations. The phenotype data set contains information on demographics (e.g., sex and age) and clinical measurements (e.g., height,

weight, blood pressure, hypertensive status, diabetic status, etc.). Furthermore, it includes genotype data from the three generations with over 900 known familial relationships, in which Affymetrix performed dense SNP genotyping using approximately 550000 SNPs (GeneChip® Human Mapping 500K Array Set and 50K Human Gene Focused Panel) in the three generations of subjects (Cupples et al., 2009). We considered 6 979 subjects with known pedigrees. Among them, 2 050 subjects in 460 pedigrees are considered for our two-stage analysis, in which the number of subjects per family ranges from 2 to 101. Moreover, a total of 467 773 SNPs on 22 autosomes will be tested for the association.

In this study, we are interested in the genetic association with respect to four CVD-related longitudinal traits: systolic blood pressure (SBP), high-density lipoprotein (HDL) cholesterol level, approximated low-density lipoprotein (LDL) cholesterol level, and triglyceride (TG) level. In stage 1, the four longitudinal traits were adjusted for confounding factors (listed in Table 10 under the column named ‘Covariate’) and were transformed if necessary so that the residuals were approximately normally distributed. To select relevant covariates for each longitudinal trait, a function called ‘bfFixefLMER_F.fnc’ found in the ‘LMERConvenienceFunctions’ package in R (Tremblay et al., 2015) is used. With the inclusion of only the selected significant covariates, a linear mixed-effect model is fit to each longitudinal trait to obtain a predicted subject-specific effect for each subject. In stage 2, we simultaneously test the association between each SNP and four predicted subject-specific effects corresponding to the four longitudinal traits. Individual tests between each SNP and each predicted subject-specific effect for each longitudinal trait are also performed for comparison.

4.2. Results

In the first step, nine potential covariates, as listed in the first column of Table 10, are considered for each of the longitudinal traits. The backward selection function in the ‘LMERConvenienceFunction’ package in R (Tremblay et al., 2015) selected different sets of covariates that have significant effects on different longitudinal traits. The selected covariates for each longitudinal trait are then included in the GLMM so the fixed effects for the selected covariates are estimated, and the subject-specific genetic effect (γ_{ijk}) and the family-specific random effect (Γ_{ik}) are predicted.

Table 10 summarizes the selected covariates for each longitudinal trait. For each selected covariate for a given longitudinal trait, the approximated upper-bound p -value is reported. The estimate of its fixed effect $\hat{a}_{k\ell}$ and the associated standard error from fitting the GLMM are also reported in Table 10. The excluded covariates in the fitted GLMMs are indicated by a dash ‘—’ in Table 10. The time-invariant covariates, sex (*Sex*) and diabetes status (*Diabetes*), are strongly significant for all traits, with p -values being nearly zero for all traits (except for the diabetes status associated with LDL for which p -value = 8.98×10^{-3}). Here, diabetes status is defined as the occurrence of diabetes at any time during the study. The time-varying covariates, BMI (*BMI*) and smoking status (*Smoke*), are strongly significant for all traits. Note that smoking status is a categorical variable with three levels: non-smoker as the reference level, former smoker, and current smoker. So, there are two coefficients $a_{k\ell}^f$ and $a_{k\ell}^c$ for the levels of former smoker and current smoker, respectively, to be estimated.

Age (*Age*) and number of ounces of equivalent alcohol consumed per week (*Alcohol*) are found to have significant effects on the $\log(\text{SBP})$, $\log(\text{HDL})$, and $\log(\text{TG})$. Number of cigarettes smoked per day (*Cigarettes*) is found to be significant for $\log(\text{HDL})$, LDL, and $\log(\text{TG})$. Cholesterol treatment (*Cholesterol* R_x) is significant for $\log(\text{SBP})$, LDL, and $\log(\text{TG})$, and hypertensive treatment (*Hypertension* R_x) is significant for $\log(\text{HDL})$ and $\log(\text{TG})$.

In the second stage, we simultaneously test the association between each SNP and all predicted subject-specific effects, where $\hat{\gamma}_1$, $\hat{\gamma}_2$, $\hat{\gamma}_3$, and $\hat{\gamma}_4$ correspond to the longitudinal traits $\log(\text{SBP})$, $\log(\text{HDL})$, LDL, and $\log(\text{TG})$, respectively. We also test the association between each SNP and the predicted subject-specific effect for each trait individually. SNPs with p -values less than 1×10^{-5} from the simultaneous test are listed in Table 11. In addition, Table 11 also reports the significance level of association with these SNPs when they are tested with each trait individually. The SNP rs3776779 is an intron variant within the *FAM174A* gene. The *FAM174A* gene has been recently recognized as one of six new candidate genes for its regulatory role in cholesterol homeostasis. Re-localization of the protein, FAM174A, to alternative organelles under reduced cholesterol levels resembled a key feature of other known regulators of cellular cholesterol homeostasis (Blattmann et al., 2013). This SNP is found to be strongly significant in the simultaneous test with the smallest p -value of 2.22×10^{-16} and is also confirmed to be significantly associated with the LDL trait with a p -value of 4.44×10^{-15} . The ten SNPs on chromosome 8 located around the region of 19.9 Mb are found by the simultaneous association test to have strong significant associations with at least one of the four traits. These SNPs are located within or close to the *LPL* gene that encodes lipoprotein lipase enzyme (Andreotti et al., 2009) and has dual functions as a triglyceride hydrolase and a ligand factor for receptor-mediated lipoprotein uptake. Results from individual tests show that all of these SNPs are significantly associated with TG and HDL traits. Note that their p -values based on the union of individual tests are consistently less than the p -values obtained from the simultaneous tests. Table 11 provides the confirmations of the associated SNPs based on other literatures. It is important note that the p -values for the individual tests listed in Table 11 have been adjusted via the Bonferroni procedure for multiple testing.

5. Discussion

In this manuscript, a two-stage method for analyzing the association with multiple longitudinal traits and particularly, for analyzing data from samples of related subjects, is proposed. In the first stage, a three-level nested mixed effects model is used to analyze each longitudinal trait. In the three-level mixed effects model, repeated measurements (level 1) are nested within subjects (level 2) and subjects are nested within families (level 3). While the fixed effects for confounding covariates are included in the model, the random effects at the subject and family levels are also included. The subject level random effect is interpreted as the unobserved subject-specific genetic effects that contribute to the variation of the longitudinal trait. The family level random effect is interpreted as the unobserved common environmental factors shared among family members that explain part of the variation of the longitudinal trait. In most situations when a mixed effects model is used to analyze longitudinal data, random effects are treated as nuisance parameters that account for the

intra-correlation among repeated measurements while the estimation of the fixed effects of the covariates of interest are of main concern. In our method, the focus is also on the prediction of the subject-specific random effects. In the second stage, a generalized quasi-likelihood scoring approach is proposed to simultaneously test the association between a given SNP and multiple subject-specific random effects arising from multiple longitudinal traits.

In the second stage, treating the observed allele frequency of each subject as the response and the subject-specific random effects as the covariates allows us to simultaneously test more than one trait. Results from both the simulation studies and real analysis show that the proposed method is more powerful for detecting genetic pleiotropy when compared to the conventional approaches of taking the consensus of individual tests. In addition, when analyzing samples of related subjects, such as family data, the GQLSM allows us to adjust for the correlation of observed genotypes among subjects due to relatedness among subjects from the same family.

In many situations, GWAS are conducted to screen the genome for a set of most relevant or important SNPs that are associated with the traits of interest. When multiple traits are studied, the simultaneous test would provide an overall significance level for the association with all traits. This makes the ranking of the significance level of the SNPs much easier than the consensus of individual tests, as individual tests only provide the significance level of an SNP for each individual trait. For example, the results from the real analysis of the GAW16 FHS data show that SNP rs780094 on chromosome 2 is strongly significant for TG (p -value = 4.93×10^{-7}) and SNP rs263 on chromosome 8 is moderately significant for HDL (p -value = 3.28×10^{-4}) and TG (p -value = 3.02×10^{-3}) by the individual tests. In this case, it is difficult to discern from the consensus of individual tests which SNP is of more concern and should have higher priority for further investigation. However, the results from the simultaneous test better answer this question, SNP rs780094 would be more significant and thus more worthy to be further investigated than SNP rs263 because rs780094 has a more significant p -value (1.65×10^{-12}) than rs263 (p -value = 2.69×10^{-6}) in the simultaneous test.

Our proposed method opens several avenues for future research. Missing data are common in longitudinal studies. For example, in a randomization clinical trial a participant may miss a particular examination for various different reasons, such that all measurements at that time point are missed. Moreover, some participants might drop out of the clinical trials for different reasons. In general, three mechanisms – missing complete at random (MCAR), missing at random (MAR), and missing not at random (MNAR) – generate missing data. Under the assumption of the MCAR mechanism, the mixed effects model in step 1 is still applicable. However, when this missing assumption is violated, typically when missing is under the MNAR assumption, using the mixed effects model to analyze such data is no longer appropriate. Therefore, methods of handling missing data under different missing mechanism assumptions are worth investigating to obtain more precise predictions of the subject-specific effects as well as better estimates of fixed effects of covariates.

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Table 1

Effects of SNPs on longitudinal traits for Studies 1 and 2.

	Study 1			Study 2		
	X_1	X_2	X_3	X_1	X_2	X_3
G_1	$b_{11} = 0.25$	$b_{21} = 0.25$	$b_{31} = 0.45$	$b_{11} = 0.25$	$b_{21} = 0.2$	$b_{31} = 0.45$
G_2	$b_{12} = 0.5$	$b_{22} = 0.55$	$b_{32} = 0.65$	$b_{12} = 0.58$	$b_{22} = 0$	$b_{32} = 0.66$
G_3	$b_{13} = 0.2$	$b_{23} = 0.15$	$b_{33} = 0.3$	$b_{13} = 0$	$b_{23} = 0.16$	$b_{33} = 0$
G_4	$b_{14} = 0.2$	$b_{24} = 0.2$	$b_{34} = 0.2$	$b_{14} = 0.24$	$b_{24} = 0.21$	$b_{34} = 0$
G_5	$b_{15} = 0.25$	$b_{25} = 0.25$	$b_{35} = 0.25$	$b_{15} = 0$	$b_{25} = 0$	$b_{35} = 0.36$

Mean and standard error of fixed-effect estimates using GLMMs and based on over 1 000 simulation replicates for sample sizes of $n = 300$, 500, and 1 000.

Table 2

Trait k	Fixed Effect $a_{k\ell}$	Study 1		Study 2	
		$\hat{a}_{k\ell}^{\dagger}$	$SE(\hat{a}_{k\ell})^{\dagger}$	$\hat{a}_{k\ell}^{\ddagger}$	$SE(\hat{a}_{k\ell})^{\ddagger}$
$n = 300$					
1	$a_{11} = 0.3$	0.300	0.058	0.301	0.057
	$a_{12} = 0.5$	0.500	0.003	0.500	0.003
2	$a_{21} = 0.2$	0.198	0.058	0.201	0.057
	$a_{22} = -0.3$	-0.300	0.003	-0.300	0.003
3	$a_{31} = -1.6$	-1.608	0.150	-1.608	0.153
	$a_{32} = 0.06$	0.060	0.007	0.060	0.007
$n = 500$					
1	$a_{11} = 0.3$	0.303	0.045	0.300	0.044
	$a_{12} = 0.5$	0.500	0.002	0.500	0.002
2	$a_{21} = 0.2$	0.201	0.045	0.200	0.044
	$a_{22} = -0.3$	-0.300	0.002	-0.300	0.002
3	$a_{31} = -1.6$	-1.609	0.115	-1.605	0.118
	$a_{32} = 0.06$	0.060	0.006	0.060	0.006
$n = 1000$					
1	$a_{11} = 0.3$	0.299	0.032	0.299	0.031
	$a_{12} = 0.5$	0.500	0.002	0.500	0.002
2	$a_{21} = 0.2$	0.201	0.032	0.200	0.031
	$a_{22} = -0.3$	-0.300	0.002	-0.300	0.002

Trait k	Fixed Effect $a_{k\ell}$	Study 1		Study 2	
		$\hat{a}_{k\ell}^{\dagger}$	SE($\hat{a}_{k\ell}^{\dagger}$)	$\hat{a}_{k\ell}^{\ddagger}$	SE($\hat{a}_{k\ell}^{\ddagger}$)
3	$a_{31} = -1.6$	-1.601	0.082	-1.606	0.083
	$a_{32} = 0.06$	0.060	0.004	0.060	0.004

† and ‡ are mean values of $\hat{a}_{k\ell}$ and SE($\hat{a}_{k\ell}$) over 1000 simulation replicates, respectively, where $k = 1, 2, 3$ and $\ell = 1, 2$.

Table 3

Type I error rate assessments based on 10000 simulation replicates in each of the two studies for sample sizes of $n = 300, 500$, and 1000 .

	$\alpha_T^{\dagger}$	Individual Tests			Simultaneous Test	
		X_1	X_2	X_3	Union	
$n = 300$						
Study 1	0.05	0.0172	0.0187	0.0171	0.0516	0.0526
	0.01	0.0032	0.0044	0.0039	0.0112	0.0117
	0.001	0.0004	0.0006	0.0007	0.0016	0.0021
Study 2	0.05	0.0195	0.0166	0.0179	0.0526	0.0553
	0.01	0.0045	0.0038	0.0034	0.0116	0.0129
	0.001	0.0005	0.0002	0.0005	0.0012	0.0018
$n = 500$						
Study 1	0.05	0.0182	0.0149	0.017	0.0487	0.0483
	0.01	0.0029	0.002	0.0027	0.0076	0.0092
	0.001	0.0002	0.0002	0.0005	0.0009	0.0014
Study 2	0.05	0.0175	0.0159	0.0157	0.0481	0.0498
	0.01	0.0042	0.004	0.003	0.0112	0.0112
	0.001	0.0006	0.0005	0.0003	0.0014	0.0014
$n = 1000$						
Study 1	0.05	0.0165	0.018	0.0161	0.0499	0.0506
	0.01	0.0031	0.0045	0.003	0.0106	0.0102
	0.001	0.0004	0.001	0.0003	0.0014	0.001
Study 2	0.05	0.0179	0.0128	0.0153	0.0455	0.0467
	0.01	0.0036	0.0027	0.0035	0.0097	0.0094
	0.001	0.0004	0.0002	0.0008	0.0014	0.0011

† For $\alpha_F = 0.05, 0.01, 0.001$, $\alpha = 0.01667, 0.00333, 0.00033$, respectively.

Table 4
Power comparisons based on 1000 simulation replicates in Study 1 for a sample size of $n = 300$.

	α_F^*	Individual Tests			Simultaneous Test	
		X_1	X_2	X_3	Union	
Study 1						
G_1	0.05	0.299	0.264	0.19	0.542	0.606
	0.01	0.129	0.127	0.085	0.291	0.394
	0.001	0.043	0.03	0.031	0.099	0.187
G_2	0.05	0.224	0.262	0.098	0.403	0.435
	0.01	0.132	0.155	0.052	0.248	0.302
	0.001	0.072	0.072	0.018	0.139	0.176
G_3	0.05	0.574	0.334	0.275	0.78	0.839
	0.01	0.379	0.164	0.137	0.526	0.666
	0.001	0.145	0.061	0.041	0.223	0.399
G_4	0.05	0.449	0.476	0.096	0.721	0.782
	0.01	0.279	0.282	0.025	0.482	0.582
	0.001	0.096	0.111	0.005	0.196	0.312
G_5	0.05	0.512	0.529	0.103	0.774	0.812
	0.01	0.307	0.33	0.047	0.53	0.63
	0.001	0.132	0.129	0.014	0.239	0.368
M_1	0.05	0.063	0.067	0.071	0.179	0.192
	0.01	0.016	0.018	0.021	0.054	0.079
	0.001	0.003	0.004	0.004	0.011	0.017
M_2	0.05	0.053	0.054	0.027	0.122	0.128
	0.01	0.017	0.02	0.016	0.05	0.054
	0.001	0.005	0.005	0.003	0.012	0.012
M_3	0.05	0.283	0.167	0.132	0.468	0.525

$\alpha_F^{\dagger}$	Individual Tests				Simultaneous Test
	X_1	X_2	X_3	Union	
0.01	0.134	0.078	0.045	0.228	0.293
	0.001	0.034	0.016	0.008	0.102
M_4	0.05	0.279	0.288	0.049	0.498
	0.01	0.141	0.15	0.012	0.278
	0.001	0.047	0.052	0.001	0.094
M_5	0.05	0.229	0.206	0.047	0.407
	0.01	0.098	0.092	0.011	0.184
	0.001	0.034	0.029	0.003	0.06

[†]For $\alpha_F=0.05, 0.01, 0.001, \alpha=0.01667, 0.00333, 0.00033$, respectively.

Highest powers are noted in boldface.

Table 5
Power comparisons based on 1000 simulation replicates in Study 1 for a sample size of $n = 500$.

	$\alpha_F^\dagger$	Individual Tests			Simultaneous Test	
		X_1	X_2	X_3	Union	
Study 1						
G_1	0.05	0.435	0.432	0.286	0.714	0.798
	0.01	0.25	0.255	0.124	0.47	0.619
	0.001	0.114	0.111	0.041	0.226	0.373
G_2	0.05	0.37	0.416	0.142	0.575	0.626
	0.01	0.219	0.272	0.064	0.396	0.469
	0.001	0.103	0.147	0.023	0.219	0.324
G_3	0.05	0.821	0.551	0.423	0.939	0.976
	0.01	0.648	0.331	0.243	0.794	0.9
	0.001	0.401	0.132	0.092	0.5	0.748
G_4	0.05	0.71	0.726	0.143	0.914	0.951
	0.01	0.513	0.514	0.047	0.753	0.863
	0.001	0.262	0.269	0.007	0.44	0.645
G_5	0.05	0.759	0.752	0.149	0.938	0.958
	0.01	0.564	0.562	0.063	0.805	0.891
	0.001	0.326	0.32	0.018	0.527	0.692
M_1	0.05	0.112	0.091	0.087	0.25	0.299
	0.01	0.054	0.035	0.03	0.116	0.143
	0.001	0.007	0.008	0.005	0.019	0.037
M_2	0.05	0.068	0.08	0.029	0.161	0.163
	0.01	0.021	0.028	0.01	0.057	0.061
	0.001	0.004	0.002	0.002	0.008	0.016
M_3	0.05	0.484	0.273	0.189	0.685	0.744

$\alpha_F^{\dagger}$	Individual Tests				Simultaneous Test
	X_1	X_2	X_3	Union	
0.01	0.311	0.116	0.091	0.445	0.57
	0.001	0.13	0.035	0.022	0.297
M_4	0.05	0.484	0.464	0.082	0.792
	0.01	0.289	0.269	0.017	0.59
	0.001	0.107	0.101	0.001	0.31
M_5	0.05	0.328	0.333	0.056	0.62
	0.01	0.178	0.164	0.014	0.372
	0.001	0.054	0.059	0.001	0.157

[†]For $\alpha_F=0.05, 0.01, 0.001, \alpha=0.01667, 0.00333, 0.00033$, respectively.
Highest powers are noted in boldface.

Table 6
Power comparisons based on 1000 simulation replicates in Study 1 for a sample size of $n = 1000$.

	$\alpha_F^\dagger$	Individual Tests			Simultaneous Test	
		X_1	X_2	X_3	Union	
Study 1						
G_1	0.05	0.763	0.802	0.606	0.969	0.987
	0.01	0.609	0.604	0.403	0.881	0.951
	0.001	0.368	0.355	0.193	0.624	0.85
G_2	0.05	0.613	0.702	0.276	0.827	0.872
	0.01	0.455	0.539	0.139	0.678	0.767
	0.001	0.275	0.338	0.057	0.456	0.604
G_3	0.05	0.99	0.874	0.793	1	1
	0.01	0.955	0.725	0.616	0.991	0.999
	0.001	0.847	0.479	0.358	0.929	0.994
G_4	0.05	0.961	0.959	0.313	0.997	1
	0.01	0.875	0.898	0.161	0.982	0.999
	0.001	0.716	0.742	0.056	0.904	0.976
G_5	0.05	0.98	0.975	0.37	0.995	0.999
	0.01	0.915	0.925	0.21	0.985	0.994
	0.001	0.775	0.806	0.078	0.94	0.983
M_1	0.05	0.231	0.195	0.146	0.45	0.531
	0.01	0.109	0.087	0.055	0.224	0.317
	0.001	0.043	0.027	0.016	0.083	0.134
M_2	0.05	0.104	0.135	0.037	0.239	0.247
	0.01	0.048	0.054	0.012	0.105	0.124
	0.001	0.01	0.019	0.004	0.032	0.04
M_3	0.05	0.809	0.519	0.477	0.933	0.968

$\alpha_F^{\dagger}$	Individual Tests				Simultaneous Test
	X_1	X_2	X_3	Union	
0.01	0.645	0.317	0.272	0.793	0.908
0.001	0.389	0.137	0.104	0.504	0.748
0.05	0.806	0.807	0.204	0.963	0.984
M_4	0.01	0.614	0.653	0.092	0.93
	0.001	0.358	0.393	0.019	0.773
0.05	0.665	0.646	0.145	0.879	0.915
M_5	0.01	0.447	0.437	0.056	0.785
	0.001	0.23	0.222	0.013	0.549

[†]For $\alpha_F=0.05, 0.01, 0.001, \alpha=0.01667, 0.00333, 0.00033$, respectively.

Highest powers are noted in boldface.

Table 7
Power comparisons based on 1000 simulation replicates in Study 2 for a sample size of $n = 300$.

	$\alpha_F^\dagger$	Individual Tests			Simultaneous Text	
		X_1	X_2	X_3		Union
Study 2						
G_1	0.05	0.261	0.171	0.198	0.484	0.578
	0.01	0.124	0.057	0.089	0.236	0.357
	0.001	0.039	0.011	0.032	0.077	0.165
G_2	0.05	0.318	0.026	0.104	0.359	0.359
	0.01	0.193	0.014	0.058	0.222	0.248
	0.001	0.115	0.006	0.02	0.125	0.151
G_3	0.05	0.019	0.386	0.012	0.386	0.384
	0.01	0.004	0.195	0.004	0.195	0.191
	0.001	0	0.067	0.001	0.067	0.058
G_4	0.05	0.665	0.52	0.015	0.839	0.881
	0.01	0.446	0.305	0.001	0.598	0.716
	0.001	0.22	0.127	0	0.313	0.453
G_5	0.05	0.017	0.018	0.211	0.211	0.228
	0.01	0.005	0.005	0.093	0.093	0.1
	0.001	0.001	0.001	0.026	0.026	0.03
M_1	0.05	0.065	0.05	0.061	0.163	0.184
	0.01	0.02	0.016	0.023	0.057	0.079
	0.001	0.003	0.002	0.005	0.01	0.015
M_2	0.05	0.067	0.014	0.032	0.096	0.117
	0.01	0.026	0.005	0.01	0.036	0.044
	0.001	0.01	0	0	0.01	0.012
M_3	0.05	0.02	0.191	0.02	0.191	0.229

$\alpha_F^{\dagger}$	Individual Tests				Simultaneous Test	
	X_1	X_2	X_3	Union		
0.01	0.003	0.078	0.004	0.078	0.081	
	0	0.023	0	0.023	0.018	
M_4	0.05	0.411	0.327	0.014	0.591	0.659
	0.01	0.238	0.169	0.001	0.363	0.432
	0.001	0.089	0.064	0	0.138	0.203
M_5	0.05	0.018	0.019	0.08	0.08	0.121
	0.01	0.006	0.004	0.023	0.023	0.031
	0.001	0.001	0.001	0.003	0.003	0.007

† For $\alpha_F=0.05, 0.01, 0.001, \alpha = 0.01667, 0.00333, 0.00033$, respectively.

Highest powers are noted in boldface.

Type 1 error rates are highlighted in grey.

Table 8
Power comparisons based on 1000 simulation replicates in Study 2 for a sample size of $n = 500$.

	$\alpha_F^{\dagger}$	Individual Tests			Simultaneous Test	
		X_1	X_2	X_3	Union	
Study 2						
G_1	0.05	0.471	0.302	0.323	0.699	0.804
	0.01	0.284	0.142	0.166	0.449	0.599
	0.001	0.129	0.047	0.06	0.201	0.388
G_2	0.05	0.446	0.028	0.162	0.491	0.512
	0.01	0.31	0.008	0.078	0.341	0.37
	0.001	0.185	0.003	0.032	0.196	0.228
G_3	0.05	0.02	0.629	0.017	0.629	0.614
	0.01	0.005	0.424	0.004	0.424	0.386
	0.001	0	0.2	0.001	0.2	0.168
G_4	0.05	0.894	0.802	0.02	0.984	0.992
	0.01	0.757	0.595	0.004	0.889	0.951
	0.001	0.521	0.331	0	0.663	0.82
G_5	0.05	0.015	0.02	0.381	0.381	0.389
	0.01	0.003	0.003	0.195	0.195	0.191
	0.001	0	0	0.054	0.054	0.052
M_1	0.05	0.123	0.082	0.079	0.249	0.295
	0.01	0.041	0.028	0.02	0.085	0.132
	0.001	0.009	0.006	0.001	0.016	0.039
M_2	0.05	0.092	0.022	0.025	0.111	0.151
	0.01	0.034	0.002	0.009	0.042	0.051
	0.001	0.014	0	0.002	0.016	0.017
M_3	0.05	0.019	0.306	0.02	0.306	0.331

$\alpha_F^{\dagger}$	Individual Tests				Simultaneous Test	
	X_1	X_2	X_3	Union		
0.01	0.005	0.151	0.005	0.151		0.147
	0	0.041	0.001	0.041		0.041
0.05	0.69	0.542	0.015	0.848		0.897
	0.01	0.48	0.344	0.641		0.736
	0.001	0.253	0.151	0.358		0.512
M_5	0.05	0.016	0.02	0.142	0.142	0.169
	0.01	0.001	0.003	0.049	0.049	0.059
	0.001	0	0.002	0.011	0.011	0.01

[†]For $\alpha_F=0.05, 0.01, 0.001, \alpha=0.01667, 0.00333, 0.00033$, respectively.

Highest powers are noted in boldface.

Type 1 error rates are highlighted in grey.

Table 9
Power comparisons based on 1000 simulation replicates in Study 2 for a sample size of $n = 1000$.

	α_F^+	Individual Tests			Simultaneous Test	
		X_1	X_2	X_3	Union	
Study 2						
G_1	0.05	0.795	0.537	0.549	0.949	0.979
	0.01	0.605	0.336	0.354	0.812	0.936
	0.001	0.371	0.143	0.17	0.522	0.803
G_2	0.05	0.782	0.023	0.289	0.811	0.83
	0.01	0.652	0.008	0.164	0.681	0.708
	0.001	0.464	0.001	0.068	0.486	0.533
G_3	0.05	0.02	0.921	0.02	0.921	0.908
	0.01	0.004	0.819	0.006	0.819	0.789
	0.001	0.001	0.602	0	0.602	0.522
G_4	0.05	1	0.982	0.021	1	1
	0.01	0.986	0.938	0.005	1	1
	0.001	0.943	0.818	0	0.985	0.998
G_5	0.05	0.018	0.014	0.683	0.683	0.659
	0.01	0.001	0.006	0.478	0.478	0.437
	0.001	0	0.001	0.251	0.251	0.216
M_1	0.05	0.213	0.129	0.127	0.389	0.474
	0.01	0.097	0.043	0.054	0.181	0.26
	0.001	0.022	0.01	0.011	0.042	0.1
M_2	0.05	0.152	0.017	0.058	0.191	0.229
	0.01	0.076	0.004	0.017	0.09	0.088
	0.001	0.022	0.001	0.001	0.023	0.035
M_3	0.05	0.021	0.621	0.017	0.621	0.607

$\alpha_F^{\dagger}$	Individual Tests				Simultaneous Test	
	X_1	X_2	X_3	Union		
M_4	0.01	0.005	0.403	0.003	0.403	0.372
	0.001	0	0.169	0	0.169	0.147
	0.05	0.954	0.869	0.018	0.995	0.999
	0.01	0.879	0.736	0.006	0.966	0.987
	0.001	0.693	0.509	0.001	0.83	0.937
	0.05	0.015	0.018	0.311	0.311	0.332
M_5	0.01	0.003	0.005	0.154	0.154	0.149
	0.001	0.001	0.001	0.054	0.054	0.048

[†]For $\alpha_F=0.05, 0.01, 0.001, \alpha=0.01667, 0.00333, 0.00033$, respectively.

Highest powers are noted in boldface.

Type 1 error rates are highlighted in grey.

Table 10
Fixed effect estimates and their associated standard errors (SEs) of covariates for each longitudinal trait using GLMM.

Covariate	Fixed Effect $a_{k\ell}$	Trait k			
		$\log(SBP)$	$\log(HDL)$	LDL	$\log(TG)$
Sex	Estimate	-0.0269	0.2551	-5.9117	-0.1054
	SE	0.0041	0.0089	1.2544	0.0182
	p -value †	≈ 0	≈ 0	1.66×10^{-10}	≈ 0
Diabetes	Estimate	0.0441	-0.0690	1.5096	0.0787
	SE	0.0070	0.0152	2.1588	0.0308
	p -value †	≈ 0	≈ 0	8.98×10^{-3}	≈ 0
Age	Estimate	0.0019	0.0024	—	0.0167
	SE	0.0001	0.0002	—	0.0005
	p -value †	≈ 0	9.54×10^{-10}	—	≈ 0
BMI	Estimate	0.0071	-0.0147	1.4312	0.0415
	SE	0.0004	0.0007	0.0956	0.0016
	p -value †	≈ 0	≈ 0	≈ 0	≈ 0
Smoke ‡	Estimate(a_{k5}^f)	-0.0162	-0.0036	3.2727	0.0083
		SE($\hat{a}_{k5}^f$)	0.0043	0.0086	1.2086
	Estimate(a_{k5}^c)	-0.0183	-0.0652	0.0323	0.0600
		SE($\hat{a}_{k5}^c$)	0.0046	0.0115	1.6540
	p -value †	3.53×10^{-3}	≈ 0	6.34×10^{-11}	6.04×10^{-14}

Covariate	Fixed Effect $a_{k\ell}$	Trait k			
		$\log(SBP)$	$\log(HDL)$	LDL	$\log(TG)$
<i>Alcohol</i>	Estimate	0.0030	0.0119	—	0.0050
	SE	0.0004	0.0006	—	0.0015
	p -value $\ddagger$	9.00×10^{-16}	≈ 0	—	6.32×10^{-4}
<i>Cigarettes</i>	Estimate	—	−0.0009	0.2592	0.0028
	SE	—	0.0004	0.0545	0.0009
	p -value $\ddagger$	—	1.98×10^{-2}	4.69×10^{-9}	1.62×10^{-3}
<i>Cholesterol/R_x</i>	Estimate	−0.0151	—	−39.7373	−0.1403
	SE	0.0051	—	1.1720	0.0206
	p -value $\ddagger$	3.10×10^{-3}	—	≈ 0	2.52×10^{-10}
<i>Hypertension R_x</i>	Estimate	—	−0.0214	—	0.0483
	SE	—	0.0066	—	0.0161
	p -value $\ddagger$	—	1.20×10^{-3}	—	2.77×10^{-3}

$\ddagger$ Approximate upper-bound p -values for the analysis of variance used in the backward selection of GLMMs.

$\ddagger$ The covariate *Smoke* is a categorical variable that has three levels: non-smoker (reference level), former smoker, and current smoker. So, there are two coefficients a_{k5}^f and a_{k5}^c for the levels of former smoker and current smoker, respectively.

Table 11

Most significant SNPs (p -values $< 10^{-5}$) based on simultaneous tests.

SNP [†]	Ch	Location (Mb)	Gene(s)	<i>p</i> -value			
				Individual Tests			
				‡ Trait	Union		
rs599839	1	109,624	CELSR2 PSRC1 SORT1	5,7,12,14,16,19LDL	4.98 × 10 ⁻¹¹	4.98 × 10 ⁻¹¹	1.82 × 10 ⁻⁹
rs780094	2	27,595	GCKR	5,8,12,15,18,19TG	4.93 × 10 ⁻⁷	4.93 × 10 ⁻⁷	1.65 × 10 ⁻¹²
rs3776779	5	99,925	FAM174A	2LDL	4.44 × 10 ⁻¹⁵	4.44 × 10 ⁻¹⁵	2.22 × 10 ⁻¹⁶
rs263 [‡]	8	19,857	LPL	HDL 1TG	3.28 × 10 ⁻⁴ 3.02 × 10 ⁻³	3.28 × 10 ⁻⁴	2.69 × 10 ⁻⁶
rs17410962	8	19,892	LPL	11,14,19HDL 14,19TG	2.47 × 10 ⁻⁷ 4.87 × 10 ⁻⁵	2.47 × 10 ⁻⁷	1.40 × 10 ⁻⁷
rs17489268	8	19,896	LPL	14,19TG 11,14,19HDL	9.22 × 10 ⁻⁷ 1.25 × 10 ⁻⁶	9.22 × 10 ⁻⁷	2.32 × 10 ⁻⁷
rs17411031 [‡]	8	19,897	LPL	14,19TG 11,14,18,19HDL	1.69 × 10 ⁻⁷ 7.03 × 10 ⁻⁷	1.69 × 10 ⁻⁷	5.54 × 10 ⁻⁸
rs17489282	8	19,897	LPL	11,19HDL 19TG	5.96 × 10 ⁻⁶ 5.96 × 10 ⁻⁶	5.96 × 10 ⁻⁶	1.93 × 10 ⁻⁶
rs17411126	8	19,900	LPL	14,19TG 11,14,19HDL	1.24 × 10 ⁻⁷ 1.57 × 10 ⁻⁶	1.24 × 10 ⁻⁷	6.69 × 10 ⁻⁸
rs765547 [‡]	8	19,911	LPL	14,19TG 11,14,19HDL	9.85 × 10 ⁻⁸ 5.49 × 10 ⁻⁷	9.85 × 10 ⁻⁸	3.36 × 10 ⁻⁸

SNP [†]	Ch	Location (Mb)	Gene(s)	<i>p</i> -value		Simultaneous Test
				Individual Tests		
				‡ Trait	Union	
rs11986942	8	19.912	<i>LPL</i>	<i>19TG</i>	2.06×10^{-7}	3.07×10^{-8}
				<i>11,14,19HDL</i>	1.77×10^{-6}	
rs1837842	8	19.913	<i>LPL</i>	<i>14,19TG</i>	1.56×10^{-7}	7.84×10^{-8}
				<i>11,14,19HDL</i>	1.43×10^{-6}	
rs1919484	8	19.914	<i>LPL</i>	<i>14,19TG</i>	6.40×10^{-7}	1.90×10^{-7}
				<i>4,11,14,19HDL</i>	1.23×10^{-6}	
<i>rs7067794⁹</i>	10	21.464	<i>NEBL</i>	<i>9,15TG</i>	6.90×10^{-4}	3.40×10^{-6}
<i>rs4775041^{5,12}</i>	15	56.462	<i>LIPC</i>	^{6,12,15,19} <i>HDL</i>	1.79×10^{-3}	2.77×10^{-6}
<i>rs4939883^{3,17}</i>	18	45.421	<i>ACAA2</i> <i>LIPG</i>	<i>11,14HDL</i>	7.47×10^{-4}	6.95×10^{-6}
<i>rs41377151¹⁷</i>	19	50.115	<i>APOC1</i>	<i>17LDL</i>	1.18×10^{-5}	4.13×10^{-7}

rs3776779, rs7067794, and rs41377151 are rare variants where their minor allele frequencies adjusted for the relationship among subjects are less than 0.01.

[†]Reference(s) for significant association between a given SNP and/or candidate gene(s) and correlated trait(s) either not listed under the column ‘Trait’ or not investigated in this study.

[‡]Reference(s) for confirmatory findings of significant association between a given SNP and/or candidate gene(s) and the given trait(s).

¹Andreotti et al. (2009),

²Blattmann et al. (2013),

³Browne et al. (2014),

⁴Chen et al. (2012),

⁵Hegele et al. (2009),

⁶Hodoglugil et al. (2010),

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⁷ Kleber et al. (2010),
⁸ Kozian et al. (2010),
⁹ Lieb et al. (2015),
¹⁰ Ma et al. (2010a),
¹¹ Ma et al. (2010b),
¹² Mohlke et al. (2008),
¹³ Muendlein et al. (2009),
¹⁴ Piccolo et al. (2009),
¹⁵ Rafiq et al. (2012),
¹⁶ Roslin et al. (2009),
¹⁷ Suchindran et al. (2010),
¹⁸ Wallace et al. (2008), and
¹⁹ Wang et al. (2014)