

RETROSPECTIVE VARYING COEFFICIENT ASSOCIATION ANALYSIS OF LONGITUDINAL BINARY TRAITS: APPLICATION TO THE IDENTIFICATION OF GENETIC LOCI ASSOCIATED WITH HYPERTENSION

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Many genetic studies contain rich information on longitudinal phenotypes that require powerful analytical tools for optimal analysis. Genetic analysis of longitudinal data that incorporates temporal variation is important for understanding the genetic architecture and biological variation of complex diseases. Most of the existing methods assume that the contribution of genetic variants is constant over time and fail to capture the dynamic pattern of disease progression. However, the relative influence of genetic variants on complex traits fluctuates over time. In this study, we propose a retrospective varying coefficient mixed model association test, RVMMAT, to detect time-varying genetic effect on longitudinal binary traits. We model dynamic genetic effect using smoothing splines, estimate model parameters by maximizing a double penalized quasi-likelihood function, design a joint test using a Cauchy combination method, and evaluate statistical significance via a retrospective approach to achieve robustness to model misspecification. Through simulations we illustrated that the retrospective varying-coefficient test was robust to model misspecification under different ascertainment schemes and gained power over the association methods assuming constant genetic effect. We applied RVMMAT to a genome-wide association analysis of longitudinal measure of hypertension in the Multi-Ethnic Study of Atherosclerosis. Pathway analysis identified two important pathways related to G-protein signaling and DNA damage. Our results demonstrated that RVMMAT could detect biologically relevant loci and pathways in a genome scan and provided insight into the genetic architecture of hypertension.

1. Introduction. Genome-wide association studies (GWAS) have successfully identified thousands of susceptible loci underlying human diseases and complex traits. Many epidemiological studies, such as Framingham Heart Study (FHS) and Women’s Health Initiative (WHI), have collected and measured health conditions and phenotypic traits on study participants over the years. Such studies provide rich resources for the investigation of genetic architecture and biological variations of complex disorders. Traditional genetic association analyses on single time point measure fail to capture the phenotypic variation over time and may lose statistical power to identify disease-related variants. Thus, genetic studies with longitudinal phenotypes require powerful analytical tools for optimal analysis. Statistical methods that account for dependence structure among observations from the same subject have been developed in GWAS to make full use of longitudinal data, such as mixed effects models

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(Furlotte, Eskin and Eyheramendy (2012), Sikorska et al. (2013), Wu et al. (2019)), generalized estimating equations (GEEs) (Sitlani et al. (2015), Wu et al. (2019)), growth mixture models (Das et al. (2011), Londono et al. (2013)), and empirical Bayes models (Meirelles et al. (2013)). Most of these methods assume that genetic contribution is constant over time. However, disease development and progression is a complicated process that changes over time. Windows of susceptibility and critical periods across the lifespan exist in disease onset and development. Studies have shown that genetic influence on the trait variations fluctuates with the passage of time (Bryois et al. (2017), Chu, Li and Reimherr (2016), Gong and Zou (2012), Liu, Li and Wu (2014), Wang, Li and Huang (2008)). Modeling time-varying genetic effects is essential to identify and validate causal genetic loci that are associated with time-dependent variation of disease progression.

Varying coefficient models are a class of generalized regression models in which the coefficients are allowed to vary smoothly with the value of other variables (Hastie and Tibshirani (1993)). They are semiparametric models that explore dynamic pattern in the data to improve model fitting (Fan and Zhang (2008)), reduce model bias by specifying the coefficients as smooth nonparametric functions (Lu and Zhang (2009)), and overcome the “curse of dimensionality” in the nonparametric estimation of multiple regression problems (Eubank et al. (2004)). There are several approaches to estimate time-varying coefficients in varying coefficient models, including kernel-local polynomial smoothing (Fan and Zhang (1999), Hoover et al. (1998), Kauermann and Tutz (1999), Wu, Chiang and Hoover (1998)), polynomial spline (Huang and Shen (2004), Huang, Wu and Zhou (2002, 2004)), and smoothing spline (Chiang, Rice and Wu (2001), Hastie and Tibshirani (1993), Zhang (2004)). Other statistical methods have been developed to model dependency in longitudinal data, such as GEE (Liang and Zeger (1986)) and Gaussian copula (Joe (2015)). Models allowing for time-varying covariate effects include semiparametric regression with GEE (Lin and Carroll (2000)) and time-varying copula models (Kürüm et al. (2018, 2016)).

Varying coefficient models have been used for two types of applications in longitudinal GWAS. The first application focuses on feature selection for longitudinal outcomes with ultrahigh-dimensional predictors, such as single nucleotide polymorphisms (SNPs). As computational burden is a major concern when handling millions of SNPs simultaneously in a model, feature screening becomes an efficient solution to filter out unimportant SNPs. Feature screening in varying coefficient models has been developed based on conditional Pearson correlation (Liu, Li and Wu (2014)), extended B-splines (Fan, Ma and Dai (2014)), modified weighted least squares estimation (Chu, Li and Reimherr (2016)), and functional regression with group penalty (Marchetti-Bowick et al. (2016)) to retain important SNPs associated with continuous and binary traits (Chu et al. (2020), Xia, Yang and Li (2016)). The second application focuses on the detection of time-varying effect of quantitative trait nucleotide, such as functional GWAS (Das et al. (2011), Li et al. (2015), Ning et al. (2017)). These methods fit the model at each SNP separately and use likelihood ratio tests to determine statistical significance, thus can be computationally intensive to analyze genome-wide SNPs, especially for binary outcomes. For large GWAS score tests are popular and computationally efficient because they only fit the null model once for all SNPs. However, current score test-based methods commonly treat the effects of genetic variants as constant over time and are not able to capture the dynamic contribution to disease progression.

Motivated by a genome-wide association analysis of longitudinal measure of hypertension in the Multi-Ethnic Study of Atherosclerosis (MESA), we developed a retrospective varying coefficient mixed model association test, RVMMAT, to detect time-varying genetic effect on longitudinal binary traits. We model dynamic genetic effect using smoothing splines, estimate model parameters by maximizing a double penalized quasi-likelihood function, design a joint test using a Cauchy combination method, and evaluate significance of the test via a

retrospective approach in which genotypes are treated as random conditional on the phenotype and covariates. Retrospective association tests have been shown to be robust to the trait model misspecification and improve statistical power (Hayeck et al. (2015), Jiang, Mbatchou and McPeck (2015), Wu et al. (2019), Wu and McPeck (2018)). In RVMMAT flexible assumptions on the effect function increased power to detect genetic variants associated with dynamic traits. RVMMAT maintains correct type I error and is less sensitive to bias of parameter estimates in the phenotype model introduced by tuning parameters in penalty terms. For comparison, we also developed VMMAT, a prospective varying coefficient mixed model association test. We conducted simulation studies to evaluate the type I error and power of RVMMAT and VMMAT and compared them with the existing association methods. The results demonstrated that the retrospective varying-coefficient test had better control of type I error when the trait model was misspecified and was robust to various ascertainment schemes. Moreover, the retrospective varying-coefficient test was more powerful than the prospective test. We applied RVMMAT and VMMAT to the genome-wide association analysis of longitudinal measure of hypertension in MESA and identified hypertension-related genetic loci and pathways.

2. Methods. Suppose a binary trait is measured over time on a sample of n subjects. We have their genome-wide measures of genetic variation and a set of covariates. The covariates are allowed to be static variables, such as sex, or dynamic variables, such as body weight. Let X_{ij} and Y_{ij} , $i = 1, \dots, n$, $j = 1, \dots, m_i$, denote the p -dimensional covariate vector and the binary trait measured on subject i at time t_{ij} . Here the measurement time and length are allowed to be different for different subjects. We let $X = (X_{1,1}, \dots, X_{1,m_1}, \dots, X_{n,1}, \dots, X_{n,m_n})^T$ denote the $N \times p$ covariate matrix and $Y = (Y_{1,1}, \dots, Y_{1,m_1}, \dots, Y_{n,1}, \dots, Y_{n,m_n})^T$ denote the outcome vector of length N , where $N = \sum_{i=1}^n m_i$ is the total number of observations. We focus on the problem of testing time-varying genetic effect between a genetic variant and the longitudinal binary trait. Let G denote the genotype vector of the n subjects at the variant to be tested, where $G_i = 0, 1$, or 2 , depending on whether subject i has 0, 1, or 2 copies of minor allele at the variant.

2.1. *GLMM with varying coefficients.* We consider a generalized linear mixed model (GLMM) with varying coefficients, specified as

$$(1) \quad g(\mu_{ij}) = \gamma_0(t_{ij}) + G_i \gamma_1(t_{ij}) + X_{ij}^T \boldsymbol{\beta} + a_i + r_{ij}, \quad i = 1, \dots, n; j = 1, \dots, m_i,$$

where $\mu_{ij} = E(Y_{ij}|G_i, X_{ij}, a_i, r_{ij})$ is the mean of the response Y_{ij} at time t_{ij} for subject i , given his/her genotype, covariates, and random effects a_i and r_{ij} , $\gamma_0(t)$, and $\gamma_1(t)$ are smooth nonparametric functions of time t , representing a time-varying intercept and a time-varying genetic effect of the tested variant, $\boldsymbol{\beta}$ is the effects of the covariates, and $g(\cdot)$ is the link function. For binary traits we use the logit link function. The correlations among repeated measurements are captured by two random effects: a_i is the subject random effect, and r_{ij} is the subject-specific time-dependent random effect (Wang et al. (2017), Wu et al. (2019)). We assume that a_i are independent and $a_i \sim N(0, \sigma_a^2)$. The vector of time-dependent random effects $\mathbf{r}_i = (r_{i,1}, \dots, r_{i,m_i})$ is assumed to follow a multivariate normal distribution, $\mathbf{r}_i \sim \text{MVN}(\mathbf{0}, \sigma_r^2 \mathbf{R}_i)$, where the correlation matrix \mathbf{R}_i is modeled by an AR(1) structure in which τ is the unknown parameter. Given the random effects a_i and r_{ij} , the response Y_{ij} are assumed to be independent. When both functions $\gamma_0(t)$ and $\gamma_1(t)$ are constants, Model (1) reduces to a standard GLMM in (Wu et al. (2019)).

Following (Lin and Zhang (1999), Zhang (2004)), we estimate $\gamma_0(t)$ and $\gamma_1(t)$ by maximizing the following double penalized quasi-likelihood (DPQL) function:

$$(2) \quad \begin{aligned} l_{dp}\{\gamma_0(\cdot), \gamma_1(\cdot), \boldsymbol{\beta}, \sigma_a^2, \sigma_r^2, \tau\} = & -\frac{1}{2} \sum_{i,j} D_{ij}(Y_{ij}, \mu_{ij}) - \frac{1}{2\sigma_a^2} \mathbf{a}^T \mathbf{a} - \frac{1}{2\sigma_r^2} \mathbf{r}^T \mathbf{R}^{-1} \mathbf{r} \\ & - \frac{\lambda_0}{2} \int \{\gamma_0^{(h_0)}(t)\}^2 dt - \frac{\lambda_1}{2} \int \{\gamma_1^{(h_1)}(t)\}^2 dt, \end{aligned}$$

where $\mathbf{a} = (a_1, \dots, a_n)^T$, $\mathbf{r} = (\mathbf{r}_1, \dots, \mathbf{r}_n)^T$ are the two vectors of random effects, $\mathbf{R} = \text{diag}\{\mathbf{R}_1, \dots, \mathbf{R}_n\}$ is a block diagonal matrix, $D_{ij}(Y_{ij}, \mu_{ij}) = -2 \int_{Y_{ij}}^{\mu_{ij}} \frac{Y_{ij}-u}{u(1-u)} du$ is the conditional deviance function of binary outcome Y_{ij} , given random effects a_i and r_{ij} , λ_k ($k = 0, 1$) are tuning parameters that control the smoothness of $\gamma_k(t)$, and h_k are positive integers for the derivative order of $\gamma_k(t)$.

The maximizers for the nonparametric functions $\gamma_k(t)$ in the DPQL function (2) are smoothing splines of order $2h_k$ (Wahba (1990)). Let $0 < t_1^0 < \dots < t_m^0 < 1$ be the m ordered distinct knots of all observed time points t_{ij} ; the smoothing splines can be expressed as

$$\gamma_k(t) = \sum_{s=1}^{h_k} c_{ks} F_{ks}(t) + \sum_{l=1}^m d_{kl} V_k(t, t_l^0), \quad k = 0, 1,$$

where $F_{ks}(t)$ is a polynomial of order $s - 1$ (e.g., $F_{ks}(t) = t^{(s-1)}/(s - 1)!$, $s = 1, \dots, h_k$) and $V_k(t_1, t_2) = \frac{1}{[(h_k-1)!]^2} \int_0^1 (t_1 - u)_+^{h_k-1} (t_2 - u)_+^{h_k-1} du$ with $u_+ = \max\{u, 0\}$. We denote $\mathbf{c}_k = (c_{k,1}, \dots, c_{k,h_k})^T$, $\mathbf{d}_k = (d_{k1}, \dots, d_{km})^T$, and $\boldsymbol{\gamma}_k = (\gamma_k(t_1^0), \dots, \gamma_k(t_m^0))^T$ for $k = 0, 1$. Then $\boldsymbol{\gamma}_k$ can be expressed as

$$(3) \quad \boldsymbol{\gamma}_k = \mathbf{F}_k \mathbf{c}_k + \mathbf{V}_k \mathbf{d}_k,$$

where \mathbf{F}_k is an $m \times h_k$ matrix with its (l, s) th entry equal to $F_{ks}(t_l^0)$ and \mathbf{V}_k is a positive definite matrix with the (l, s) th entry equal to $V_k(t_l^0, t_s^0)$. Similar to equation (5) in (Zhang (2004)), the DPQL function (2) becomes

$$(4) \quad \begin{aligned} l_{dp}\{\gamma_0(\cdot), \gamma_1(\cdot), \boldsymbol{\beta}, \sigma_a^2, \sigma_r^2, \tau\} = & -\frac{1}{2} \sum_{i,j} D_{ij}(Y_{ij}, \mu_{ij}) - \frac{1}{2\sigma_a^2} \mathbf{a}^T \mathbf{a} - \frac{1}{2\sigma_r^2} \mathbf{r}^T \mathbf{R}^{-1} \mathbf{r} \\ & - \frac{\lambda_0}{2} \mathbf{d}_0^T \mathbf{V}_0 \mathbf{d}_0 - \frac{\lambda_1}{2} \mathbf{d}_1^T \mathbf{V}_1 \mathbf{d}_1. \end{aligned}$$

Following (Lin and Zhang (1999), Zhang (2004)), if we plug equation (3) into Model (1), it indicates that the maximizers of the DPQL function (4) can be obtained by fitting the GLMM representation of Model (1), expressed in a matrix form as

$$(5) \quad g(\boldsymbol{\mu}) = \mathbf{M} \mathbf{F}_0 \mathbf{c}_0 + \boldsymbol{\Delta}_G \mathbf{M} \mathbf{F}_1 \mathbf{c}_1 + \mathbf{X} \boldsymbol{\beta} + \mathbf{M} \mathbf{V}_0 \mathbf{d}_0 + \boldsymbol{\Delta}_G \mathbf{M} \mathbf{V}_1 \mathbf{d}_1 + \mathbf{B} \mathbf{a} + \mathbf{r},$$

where \mathbf{M} is an $N \times m$ incidence matrix mapping the m distinct knots t_1^0, \dots, t_m^0 to measurement time t_{ij} , with its (l, s) th entry $M_{ls} = 1$ if the l th entry of \mathbf{Y} is measured at time t_s^0 and 0 otherwise; \mathbf{B} is an $N \times n$ design matrix mapping the subject-level genotype vector \mathbf{G} to a measurement-level genotype vector $\mathbf{B} \mathbf{G}$, with its (l, i) th entry $B_{li} = 1$ if the l th entry of \mathbf{Y} is a measurement on subject i and 0 otherwise; $\boldsymbol{\Delta}_G = \text{diag}\{\mathbf{B} \mathbf{G}\} = \text{diag}\{G_1, \dots, G_1, \dots, G_n, \dots, G_n\}$ is an N -dimensional diagonal matrix of the genotypes for the n subjects; \mathbf{c}_0 and \mathbf{c}_1 are regression coefficients, and \mathbf{d}_k are random effects distributed as $\mathbf{d}_k \sim N(\mathbf{0}, \theta_k \mathbf{V}_k^{-1})$ for $k = 0, 1$, where $\theta_k = \lambda_k^{-1}$.

Model (5) is a specific implementation of the GLMM with varying coefficients in (Zhang (2004)). The smoothing parameters λ_0 and λ_1 in the DPQL function (2) are reparameterized as θ_0 and θ_1 and treated as extra variance components in addition to σ_a^2 and σ_r^2 in Model (5). Since the DPQL function (4) is the same as the log-likelihood of Model (5), our estimation of the nonparametric functions, smoothing parameters, and variance components in the DPQL function (2) can be easily obtained by fitting the working GLMM (5) using the penalized quasi-likelihood approach (Breslow and Clayton (1993)), which iteratively fits a linear mixed model to a working response variable, obtains the best linear unbiased predictors (BLUPs) of the fixed effects and the random effects, and estimates the variance components using restricted maximum likelihood (REML). The detailed algorithm to estimate the fixed effects and the random effects in Model (5) follows the procedure described in Section 3.3 of (Lin and Zhang (1999)) and can be found in the Supplementary Material (Xu et al. (2024)). The DPQL estimator $\hat{\boldsymbol{\gamma}}_k$ can be calculated as $\hat{\boldsymbol{\gamma}}_k = \mathbf{F}_k \hat{\mathbf{c}}_k + \mathbf{V}_k \hat{\mathbf{d}}_k$, which is a linear combination of the penalized quasi-likelihood estimators of the fixed effects $\hat{\mathbf{c}}_k$ and the random effects $\hat{\mathbf{d}}_k$ in Model (5).

It is well known that the penalized quasi-likelihood estimation procedure tends to underestimate the variance components for sparse binary data (Breslow and Clayton (1993), Breslow and Lin (1995), Lin and Breslow (1996)). When the response probabilities are small and the data are highly imbalanced, only limited information is available for estimating random effects and their variances and covariances, leading to a convergence to a nonpositive definite covariance matrix and biased variance component estimates (Breslow and Clayton (1993)). To improve the asymptotic performance of the penalized quasi-likelihood estimators for correlated binary data, we used a bias correction procedure (Lin and Breslow (1996), Lin and Zhang (1999), Zhang (2004)) to produce less biased variance component estimates and hence less biased nonparametric functions. The bias correction procedure is as follows: (1) use the penalized quasi-likelihood approach to estimate regression coefficients and variance components; (2) correct variance components, and (3) recalculate regression coefficients using the corrected variance components. The detailed bias correction algorithm can be found in Section 5.3 of (Lin and Breslow (1996)).

2.2. *Varying coefficient mixed model association test.* To test time-varying genetic effect between the variant and the trait, we test $H_0 : \gamma_1(t) = 0$ in Model (1), which is equivalent to test $H_0 : \mathbf{c}_1 = \mathbf{0}$ and $\theta_1 = 0$ in Model (5). The reduced GLMM under the null hypothesis specifies that

$$(6) \quad g(\boldsymbol{\mu}_0) = \mathbf{M}\mathbf{F}_0\mathbf{c}_0 + \mathbf{X}\boldsymbol{\beta} + \mathbf{M}\mathbf{V}_0\mathbf{d}_0 + \mathbf{B}\mathbf{a} + \mathbf{r},$$

where $\boldsymbol{\mu}_0 = E(\mathbf{Y}|\mathbf{F}_0, \mathbf{X}, \mathbf{d}_0, \mathbf{a}, \mathbf{r})$.

If we test $H_0 : \mathbf{c}_1 = \mathbf{0}$ under the assumption that $\theta_1 = 0$, a score test statistic can be constructed as

$$(7) \quad T_f = (\mathbf{U}_0(\mathbf{c}_1))^T [\text{Var}(\mathbf{U}_0(\mathbf{c}_1))]^{-1} \mathbf{U}_0(\mathbf{c}_1),$$

where $\mathbf{U}_0(\mathbf{c}_1) = (\Delta_G \mathbf{M}\mathbf{F}_1)^T (\mathbf{Y} - \hat{\boldsymbol{\mu}}_0)$ is the score function for \mathbf{c}_1 and $\hat{\boldsymbol{\mu}}_0 = g^{-1}(\mathbf{M}\mathbf{F}_0 \hat{\mathbf{c}}_0 + \mathbf{X}\hat{\boldsymbol{\beta}} + \mathbf{M}\mathbf{V}_0 \hat{\mathbf{d}}_0 + \mathbf{B}\hat{\mathbf{a}} + \hat{\mathbf{r}})$ is a vector of fitted values under Model (6). Given the genotype and covariates, the variance of the score $\mathbf{U}_0(\mathbf{c}_1)$ under H_0 is

$$\text{Var}(\mathbf{U}_0(\mathbf{c}_1)) = (\Delta_G \mathbf{M}\mathbf{F}_1)^T \mathbf{P} \Delta_G \mathbf{M}\mathbf{F}_1,$$

where $\mathbf{P} = \boldsymbol{\Psi}^{-1} - \boldsymbol{\Psi}^{-1} \mathbf{X}_F (\mathbf{X}_F^T \boldsymbol{\Psi}^{-1} \mathbf{X}_F)^{-1} \mathbf{X}_F^T \boldsymbol{\Psi}^{-1}$, $\mathbf{X}_F = (\mathbf{M}\mathbf{F}_0, \mathbf{X})$ and $\boldsymbol{\Psi} = \hat{\boldsymbol{\Gamma}}_0^{-1} + \hat{\boldsymbol{\theta}}_0 \mathbf{M}\mathbf{V}_0 \mathbf{M}^T + \hat{\sigma}_a^2 \mathbf{B}\mathbf{B}^T + \hat{\sigma}_r^2 \hat{\mathbf{R}}$. Here, $\boldsymbol{\Gamma} = \text{diag}\{\mu_{1,1}(1 - \mu_{1,1}), \dots, \mu_{1,m_1}(1 - \mu_{1,m_1}), \dots, \mu_{n,1}(1 - \mu_{n,1}), \dots, \mu_{n,m_n}(1 - \mu_{n,m_n})\}$ is an N -dimensional diagonal matrix, and $\hat{\boldsymbol{\Gamma}}_0$ and $\hat{\mathbf{R}}$

are Γ and \mathbf{R} evaluated under Model (6). Under the null hypothesis, the T_f test statistic has an asymptotic χ^2 distribution with h_1 degrees of freedom.

On the other hand, if we test $H_0 : \theta_1 = 0$ under the assumption that $\mathbf{c}_1 = \mathbf{0}$, a variance component score test statistic can be constructed as

$$(8) \quad T_{vc} = (\mathbf{Y} - \hat{\boldsymbol{\mu}}_0)^T \boldsymbol{\Delta}_G \mathbf{M} \mathbf{V}_1 \mathbf{M}^T \boldsymbol{\Delta}_G (\mathbf{Y} - \hat{\boldsymbol{\mu}}_0).$$

Under the null hypothesis, T_{vc} asymptotically follows a mixture of χ^2 distribution, $T_{vc} \sim \sum_{l=1}^m \xi_l \chi_{1,l}^2$, where (ξ_1, \dots, ξ_m) are the eigenvalues of the matrix $\mathbf{V}_1^{1/2} \mathbf{M}^T \boldsymbol{\Delta}_G \mathbf{P} \boldsymbol{\Delta}_G \mathbf{M} \mathbf{V}_1^{1/2}$ and $\chi_{1,l}^2$ are independent χ_1^2 variables. The p-value of T_{vc} can be evaluated by a moment-matching method (Liu, Tang and Zhang (2009)).

We propose a joint test for testing $H_0 : \mathbf{c}_1 = \mathbf{0}$ and $\theta_1 = 0$ using a Cauchy combination test (Liu and Xie (2020)) that combines the test of fixed effect, T_f , and the test of variance component, T_{vc} , which we named as Varying-coefficient Mixed Model Association Test (VMMAT). Specifically, the VMMAT test statistic is

$$(9) \quad T_{\text{VMMAT}} = \frac{1}{2} \{ \tan[(0.5 - p_f)\pi] + \tan[(0.5 - p_{vc})\pi] \},$$

where p_f and p_{vc} are p-values of T_f and T_{vc} . Under the null hypothesis, T_{VMMAT} asymptotically follows a Cauchy distribution. Its p-value can be approximated by $p_{\text{VMMAT}} = 0.5 - \arctan(T_{\text{VMMAT}})/\pi$.

The asymptotic null distributions of T_f and T_{vc} are based on the working GLMM (5) which is an equivalent representation of the GLMM with varying coefficients using smoothing splines. The null distributions of the score test statistics, T_f and T_{vc} , as well as the combined test statistic, T_{VMMAT} , depend on the tuning parameter values in the DPQL function (2), which are reparameterized and treated as extra variance components in the working GLMM (5). Correct calibration of a prospective test relies on accurate estimation of one or more variance component parameters in the trait model, which is often challenging to achieve. For correlated binary data, regression coefficients and variance components are usually estimated using the penalized quasi-likelihood approach with bias correction, and the bias-corrected estimators are expected to have an improved asymptotic performance. Even with bias correction, VMMAT may still have inadequate control of type I error. We further assessed the null distribution of VMMAT through type I error experiments in simulation studies.

2.3. Retrospective varying coefficient mixed model association test. Retrospective association tests have been shown to be robust to the trait model misspecification and improve statistical power (Hayeck et al. (2015), Jiang, Mbatchou and McPeck (2015), Wu et al. (2019), Wu and McPeck (2018)). In that follows, we introduce a new varying-coefficient test, RVM-MAT (Retrospective Varying-coefficient Mixed Model Association Test), for testing time-varying genetic effect between the variant and the trait. RVM-MAT also uses a Cauchy combination test to combine two tests: a test for $H_0 : \mathbf{c}_1 = \mathbf{0}$ under the constraint $\theta_1 = 0$ and a test for $H_0 : \theta_1 = 0$ under the constraint $\mathbf{c}_1 = \mathbf{0}$. In contrast to the two prospective tests, T_f and T_{vc} , in VMMAT, the two tests for testing fixed effect and variance component in RVM-MAT are based on a retrospective model of the genotype, given the trait and covariates, such that the analysis is less dependent on the correct specification of the phenotype model. Under the null hypothesis of no genetic effect between the variant and the trait, the quasi-likelihood model of the genotype \mathbf{G} conditional on the phenotype \mathbf{Y} and covariates \mathbf{X} can be specified as (Jiang, Zhong and McPeck (2016), Thornton and McPeck (2010), Wu et al. (2019))

$$(10) \quad E_0(\mathbf{G}|\mathbf{Y}, \mathbf{X}) = 2p\mathbf{1}_n, \quad \text{Var}_0(\mathbf{G}|\mathbf{Y}, \mathbf{X}) = \sigma_g^2 \boldsymbol{\Phi},$$

where p is the minor allele frequency (MAF) of the tested variant, $\mathbf{1}_n$ is a vector of length n with every element equals to 1, σ_g^2 is an unknown variance parameter, and Φ is an $n \times n$ genetic relationship matrix (GRM), representing the overall genetic similarity between samples due to population structure, which can be estimated using genome-wide data.

When we test $H_0 : \mathbf{c}_1 = \mathbf{0}$ under the assumption that $\theta_1 = 0$, the same score function $U_0(\mathbf{c}_1)$ is considered. Because the vector of null phenotypic residuals $\mathbf{Y} - \hat{\boldsymbol{\mu}}_0$, obtained by fitting Model (6), is orthogonal to the column space of $\mathbf{X}_F = (\mathbf{M}\mathbf{F}_0, \mathbf{X})$, then the null mean model of \mathbf{G} in equation (10) ensures that

$$E_0(U_0(\mathbf{c}_1)|\mathbf{Y}, \mathbf{X}) = E_0[(\Delta_G \mathbf{M}\mathbf{F}_1)^T (\mathbf{Y} - \hat{\boldsymbol{\mu}}_0)|\mathbf{Y}, \mathbf{X}] = 2p(\mathbf{M}\mathbf{F}_1)^T (\mathbf{Y} - \hat{\boldsymbol{\mu}}_0) = \mathbf{0},$$

if $h_1 \leq h_0$. In practice, we commonly use smoothing splines of the same order for $\gamma_0(t)$ and $\gamma_1(t)$ in Model (1). Thus, we consider the score function $U_0(\mathbf{c}_1)$ and construct score test statistic under the retrospective Model (10), given by

$$(11) \quad T_f^R = (U_0(\mathbf{c}_1))^T [\text{Var}_0(U_0(\mathbf{c}_1)|\mathbf{Y}, \mathbf{X})]^{-1} U_0(\mathbf{c}_1).$$

Here the variance of $U_0(\mathbf{c}_1)$ is evaluated by

$$\begin{aligned} \text{Var}_0(U_0(\mathbf{c}_1)|\mathbf{Y}, \mathbf{X}) &= \text{Var}_0((\Delta_G \mathbf{M}\mathbf{F}_1)^T (\mathbf{Y} - \hat{\boldsymbol{\mu}}_0)|\mathbf{Y}, \mathbf{X}) \\ &= \text{Var}_0((\Delta_R \mathbf{M}\mathbf{F}_1)^T \mathbf{B}\mathbf{G}|\mathbf{Y}, \mathbf{X}) \\ &= \hat{\sigma}_g^2 (\Delta_R \mathbf{M}\mathbf{F}_1)^T \mathbf{B}\Phi\mathbf{B}^T \Delta_R \mathbf{M}\mathbf{F}_1, \end{aligned}$$

where $\Delta_R = \text{diag}\{\mathbf{Y} - \hat{\boldsymbol{\mu}}_0\} = \text{diag}\{(Y_{1,1} - \hat{\mu}_{0;1,1}), \dots, (Y_{1,m_1} - \hat{\mu}_{0;1,m_1}), \dots, (Y_{n,1} - \hat{\mu}_{0;n,1}), \dots, (Y_{n,m_n} - \hat{\mu}_{0;n,m_n})\}$ is an N -dimensional diagonal matrix of the phenotypic residuals. Under Hardy–Weinberg equilibrium, the variance of the genotype is estimated by $\hat{\sigma}_g^2 = 2\hat{p}(1 - \hat{p})$, where \hat{p} is the sample MAF of the tested variant. Under the null hypothesis, the T_f^R test statistic has an asymptotic χ^2 distribution with h_1 degrees of freedom.

If we test $H_0 : \theta_1 = 0$ under the assumption that $\mathbf{c}_1 = \mathbf{0}$, a retrospective variance component score test statistic under Model (10) can be constructed as

$$(12) \quad T_{vc}^R = (\mathbf{B}\mathbf{G})^T \Delta_R \mathbf{M}\mathbf{V}_1 \mathbf{M}^T \Delta_R \mathbf{B}\mathbf{G} = (\mathbf{Y} - \hat{\boldsymbol{\mu}}_0)^T \Delta_G \mathbf{M}\mathbf{V}_1 \mathbf{M}^T \Delta_G (\mathbf{Y} - \hat{\boldsymbol{\mu}}_0),$$

which has the same form as the prospective variance component score test statistic T_{vc} . However, under the null hypothesis, given the trait and covariates, T_{vc}^R asymptotically follows a mixture of χ^2 distribution, $T_{vc}^R \sim \sum_{l=1}^m \zeta_l \chi_{l,l}^2$, where $(\zeta_1, \dots, \zeta_m)$ are the eigenvalues of the matrix $\hat{\sigma}_g^2 \mathbf{V}_1^{1/2} \mathbf{M}^T \Delta_R \mathbf{B}\Phi\mathbf{B}^T \Delta_R \mathbf{M}\mathbf{V}_1^{1/2}$.

The RVMMAT test statistic is defined by combining the two retrospective score test statistics, T_f^R and T_{vc}^R , expressed as

$$(13) \quad T_{RVMMAT} = \frac{1}{2} \{ \tan[(0.5 - p_f^R)\pi] + \tan[(0.5 - p_{vc}^R)\pi] \},$$

where p_f^R and p_{vc}^R are p-values of T_f^R and T_{vc}^R . Under the null hypothesis, T_{RVMMAT} asymptotically follows a Cauchy distribution.

In retrospective association analysis, the genotype at a tested variant is treated as random, and the analysis is performed, conditional on the phenotype and covariates. The retrospective score test statistics, T_f^R and T_{vc}^R , are constructed based on retrospective analysis that considers the conditional distribution of the genotype, given phenotype and covariate information, under the null hypothesis of no association. Therefore, their null distributions rely on a genotypic model and would be expected to be more sensitive to misspecification of the genotype model under the null hypothesis. While T_f^R and T_{vc}^R make use of variance component parameters in the trait model, they are less sensitive to whether or not these parameters are well

estimated. As a result, the combined test statistic, T_{RVMMAT} , is robust to bias of parameter estimates in the phenotype model introduced by tuning parameters in penalty terms. We also assessed the null distribution of RVMMAT through type I error experiments in simulation studies.

3. Simulation studies. We conducted simulation studies to assess the type I error and power of VMMAT and RVMMAT, and compared them to a Gaussian copula method with weighted scores that allows for heterogenous genetic effect (Nikoloulopoulos, Joe and Chaganty (2011)) and the two association tests that assume constant genetic effect, GMMAT (Chen et al. (2016)) and RGMMAT (Wu et al. (2019)). In all simulations VMMAT and RVMMAT were implemented with cubic smoothing splines. The Gaussian copula method was implemented with a binomial marginal model with the logit link function and an AR(1) structure in the Gaussian copula correlation matrix (Nikoloulopoulos, Joe and Chaganty (2011)). VMMAT and RVMMAT are designed to detect time-varying genetic effect between a genetic variant and the longitudinal binary trait. Because we test one variant at a time, these methods tend to have limited power for rare variants and are more appropriate for common variants. The performance of all methods was evaluated on common variants in simulation studies. We considered two trait models and three ascertainment schemes to evaluate the robustness of VMMAT and RVMMAT in the presence of model misspecification and ascertainment.

3.1. *Simulation settings.* To generate genotypes, we first simulated 10,000 chromosomes over a one Mb region using a coalescent model to mimic the recombination rates and linkage disequilibrium (LD) pattern of the European population (Schaffner et al. (2005), Shlyakhter, Sabeti and Schaffner (2014)). We then randomly selected 1000 noncausal SNPs with MAF > 0.05. In addition, we simulated two causal SNPs that were assumed to influence the trait value with epistasis. In each simulation setting, we generated 1000 sets of binary phenotypes at five time points for a given sample size. In the type I error experiments for each phenotype dataset, we tested the time-varying genetic effect at the 1000 noncausal SNPs. In total, 10^6 test results across 1000 phenotype datasets were used for the type I error assessment. In the power simulations, we tested time-varying genetic effect at the first of the two causal SNPs and evaluated power using 1000 simulated phenotype datasets. In all tests considered, the genotypes at the untested SNPs were not included as covariates in the model.

We simulated binary phenotypes under two types of trait models at five time points in which the two unlinked causal SNPs were assumed to influence phenotype through an epistatic interaction. The first type is a logistic mixed model, specified by

$$\begin{aligned}
 Y_{ij} | \mathbf{X}_{ij}, G_{i(1)}, G_{i(2)}, a_i, r_{ij} &\sim \text{Bernoulli}(\mu_{ij}), \\
 \text{logit}(\mu_{ij}) &= -1.9 + 0.2j + \gamma_1(t_{ij}) \mathbb{I}_{\{G_{i(1)} > 0, G_{i(2)} > 0\}} \\
 &\quad + 0.5X_{ij(1)} + 0.5X_{i(2)} + a_i + r_{ij}, \\
 i &= 1, \dots, n; \quad j = 1, \dots, 5,
 \end{aligned}$$

where $\gamma_1(t_{ij})$ is a function encoding the effect of the causal SNPs, $G_{i(1)}$ and $G_{i(2)}$ are the genotypes of subject i at the two causal SNPs, $\mathbb{I}_{\{G_{i(1)} > 0, G_{i(2)} > 0\}}$ is an indicator function that takes value 1 when subject i has at least one copy of the minor allele at both causal SNPs, $X_{ij(1)}$ is a continuous, time-varying covariate generated from a multivariate normal distribution with a compound symmetry correlation matrix where the correlation is 0.5, $X_{i(2)}$ is a binary, time-invariant covariate taking values 0 or 1 with a probability of 0.5, and a_i and r_{ij} are the subject-level time-independent and time-dependent random effects, respectively. We assumed $a_i \sim N(0, \sigma_a^2)$ and $\mathbf{r}_i = (r_{i1}, \dots, r_{i5}) \sim \text{MVN}(\mathbf{0}, \sigma_r^2 \mathbf{R})$, where \mathbf{R} is a 5×5 correlation matrix specified by the AR(1) structure with a correlation coefficient τ . The two

causal SNPs were assumed to be unlinked with MAFs 0.1 and 0.5, respectively. The variance components were set to $\sigma_a^2 = \sigma_r^2 = 0.64$ and $\tau = 0.7$, the same values used by (Wu et al. (2019)).

The second type of trait model is a liability threshold model in which an underlying continuous liability determines the binary outcome value based on a threshold. Specifically, the phenotype Y_{ij} is determined by

$$Y_{ij} = 1 \quad \text{if } L_{ij} > 0,$$

$$\text{with } L_{ij} = -1.8 + 0.2j + \gamma_1(t_{ij})\mathbb{I}_{\{G_{i(1)} > 0, G_{i(2)} > 0\}} + 0.5X_{ij(1)} + 0.5X_{i(2)} + a_i + r_{ij} + e_{ij},$$

where L_{ij} is the underlying liability for subject i at time t_{ij} , and $e_{ij} \sim N(0, \sigma_e^2)$ represents independent noise, with $\sigma_e^2 = 1.96$. All other parameters are the same as those in the logistic mixed model.

In both trait models, we specified the intercept as a linear function of time $t_{ij} = j$, and the genetic effect as a logistic function $\gamma_1(t_{ij}) = \frac{\gamma}{\{1 + \gamma \exp(8 - 2.4t_{ij})\}}$ (Gong and Zou (2012)). For the type I error assessment, the effect of the causal SNPs was set to $\gamma = 0.6$ in $\gamma_1(t_{ij})$. For the power evaluation, we considered a range of values for γ , where $\gamma = 0.6, 0.63, 0.66$, and 0.69 . At the given parameter values, the prevalence of the event of interest ranges from 23.68% to 40.56% over time. The proportion of the phenotypic variance explained by the two causal SNPs ranges from 0.01% to 2.99% in the logistic mixed model and from 0.01% to 1.36% in the liability threshold model. This range of variation explained is commonly seen in GWAS.

We considered three sampling designs as in (Wu et al. (2019)). In the “random” sampling, samples contain 2000 subjects randomly selected from the population regardless of their phenotypes. In the “baseline” sampling, samples contain 1000 case subjects and 1000 control subjects based on their outcome value at baseline only. In the “sum” sampling, subjects were stratified into three strata based on the total count of events of the subject over time, where subjects in stratum 1 never experienced the event of interest, that is, $\sum_j Y_{ij} = 0$, subjects in stratum 2 sometimes experienced the event, that is, $0 < \sum_j Y_{ij} < n_i$, and subjects in stratum 3 always experienced the event, that is $\sum_j Y_{ij} = n_i$. We oversampled subjects with response variation over the course of the study and selected 100, 1800, and 100 subjects from the three strata (Schildcrout et al. (2018)).

3.2. Simulation results. To assess type I error, we tested time-varying genetic effect at unlinked and unassociated SNPs. Empirical type I error was calculated as the proportion of simulations in which the p-value of the SNP is less than the nominal level α , for $\alpha = 0.01, 0.001$, and 0.0001 . Table 1 gives the empirical type I error rates of RVMMAT and VMMAT, based on 10^6 replicates, under two trait models and three sampling designs. The 95% confidence interval of a nominal level α was calculated as $\alpha \pm 1.96\sqrt{\alpha(1 - \alpha)}/10^6$. In most simulations the type I error of RVMMAT was within the 95% confidence interval of the nominal levels. In contrast, the type I error of VMMAT in all simulation settings was much lower than the nominal level when $\alpha = 0.01, 0.001$, and 0.0001 . It is well recognized that the DPQL approach underestimates variance components when data are sparse, such as binary data (Lin and Zhang (1999), Zhang (2004)). Even with bias correction, parameters estimated from the DPQL function with penalty terms depend on the tuning parameter values. Thus, the prospective variance of the score $U_0(\mathbf{c}_1)$ tends to be overestimated, producing a conservative test statistic. However, the retrospective variance of the score $U_0(\mathbf{c}_1)$ is less sensitive to bias of parameter estimates in the phenotype model introduced by tuning parameters in penalty terms so that the test statistic is less biased. These results suggest that the retrospective RVMMAT test had much better control of type I error and was robust to trait

TABLE 1
Empirical type I error of RVMMAT and VMMAT, based on 10^6 replicates

Test	Level	Logistic Mixed Model			Liability Threshold Model		
		Random	Baseline	Sum	Random	Baseline	Sum
RVMMAT	0.01	9.90×10^{-3}	1.01×10^{-2}	9.97×10^{-3}	1.02×10^{-2}	9.70×10^{-3}	1.00×10^{-2}
	0.001	9.52×10^{-4}	9.92×10^{-4}	9.17×10^{-4}	1.04×10^{-3}	1.00×10^{-3}	1.03×10^{-3}
	0.0001	9.80×10^{-5}	1.08×10^{-4}	9.40×10^{-5}	1.00×10^{-4}	1.01×10^{-4}	1.13×10^{-4}
VMMAT	0.01	5.77×10^{-3}	6.45×10^{-3}	6.91×10^{-3}	5.73×10^{-3}	6.26×10^{-3}	7.20×10^{-3}
	0.001	4.34×10^{-4}	5.14×10^{-4}	5.33×10^{-4}	4.73×10^{-4}	5.10×10^{-4}	6.68×10^{-4}
	0.0001	3.70×10^{-5}	4.40×10^{-5}	5.70×10^{-5}	2.90×10^{-5}	5.00×10^{-5}	4.60×10^{-5}

The 95% confidence interval of a nominal level α was calculated as $\alpha \pm 1.96\sqrt{\alpha(1-\alpha)/10^6}$. Specifically, the 95% confidence intervals are $(9.80 \times 10^{-3}, 1.02 \times 10^{-2})$ for $\alpha = 0.01$, $(9.38 \times 10^{-4}, 1.06 \times 10^{-3})$ for $\alpha = 0.001$, and $(8.04 \times 10^{-5}, 1.20 \times 10^{-4})$ for $\alpha = 0.0001$. Rates outside of the 95% confidence interval are in bold.

model misspecification and ascertainment, whereas the prospective VMMAT test was overly conservative.

To compare power, we considered four parameter values for γ to determine time-varying genetic effect at the two causal SNPs and tested between the trait and the first causal SNP. Empirical power was calculated at the significance level 10^{-3} , based on 1000 replicates. Figure 1 demonstrates the power results of the five methods, RVMMAT, VMMAT, Copula, RGMMAT, and GMMAT, under two trait models and three sampling designs. In all simulation settings, the two varying-coefficient tests consistently had higher power than the association tests assuming constant genetic effect. The Gaussian copula method with heterogenous genetic effect had lower power than RVMMAT and VMMAT, while performed better than

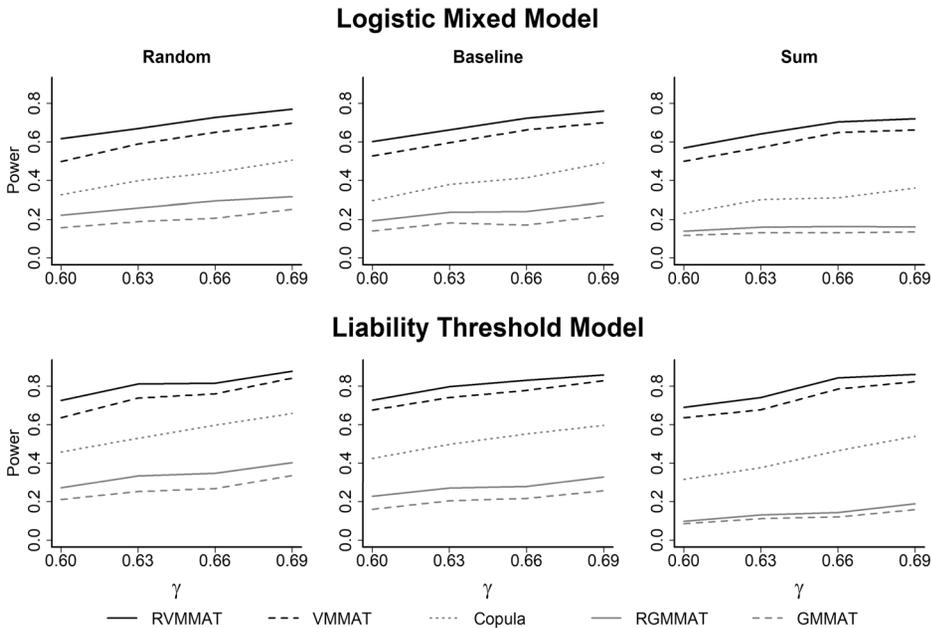


FIG. 1. Empirical power of RVMMAT, VMMAT, Copula, RGMMAT and GMMAT. Power is based on 1000 replicates at five time points with $\alpha = 10^{-3}$. In the upper panel, trait is simulated under the logistic mixed model; in the lower panel, trait is simulated under the liability threshold model. Power results are demonstrated in samples of 2000 individuals according to three ascertainment schemes: random, baseline, and sum.

RGMMAT and GMMAT. Moreover, within the same type of tests, the retrospective test was more powerful than the prospective test. Both RVMMAT and VMMAT had similar power across the three sampling designs. In contrast, Copula, RGMMAT, and GMMAT had lower power under the sum sampling in both trait models. The power gain of the varying-coefficient tests was more prominent over the association tests, assuming constant genetic effect in the presence of ascertainment. These results suggest that RVMMAT was the most powerful test and outperformed the association tests, assuming constant genetic effect.

4. Application to MESA data. We illustrated the utility of our proposed methods by analyzing a GWAS dataset of hypertension in MESA (Bild et al. (2002)). MESA is a large longitudinal study of subclinical cardiovascular disease (CVD) whose primary objective is to understand the pathogenesis of atherosclerosis and other CVD. We analyzed longitudinal hypertension assessed at five time points on 6429 participants. Among them 39.3% are white, 26.1% are African-American, 22.5% are Hispanic, and 12.1% are Asian. The proportion of case subjects at each time point ranges from 44.6% ($n = 2864$) to 59.5% ($n = 2608$), and the missing rate of the hypertension outcome at each time point ranges from 0 to 31.6%.

Samples were genotyped using the Affymetrix Human SNP Array 6.0. After data cleaning, there were 6428 subjects available for genotype imputation. We applied IMPUTE2 (Howie, Donnelly and Marchini (2009)) for imputation, using the 1000 Genomes Phase 3 data as a reference panel. Subjects who did not meet either of the following criteria were excluded: (1) proportion of successfully imputed SNPs $> 95\%$ and (2) empirical inbreeding coefficient < 0.05 . Based on the above criteria, 6424 subjects were retained in the downstream analysis, with 3057 males and 3367 females, of whom 2527 are white, 1673 are African American, 1449 are Hispanic, and 775 are Asian. There are 2227 subjects who had no hypertension during the study period, 1807 subjects who were sometimes hypertensive, that is, exhibited response variation, and 2390 subjects who were always hypertensive over the course of the study. We then tested Hardy–Weinberg equilibrium at each SNP within each population. SNPs met all of the following quality-control conditions were included in the analysis: (1) call rate $> 95\%$, (2) Hardy–Weinberg χ^2 statistic p-value $> 10^{-6}$, and (3) MAF $> 1\%$. Taken together, a final set of 6,155,404 SNPs were examined in the downstream analysis.

4.1. Analysis of time-varying genetic effect. Our goal in the MESA data is to detect time-varying genetic effect on longitudinal measure of hypertension. The null hypothesis is no genetic effect vs. the alternative hypothesis that allows for time-varying effects on hypertension. We performed genome-wide tests of time-varying genetic effect on hypertension using RVMMAT and VMMAT with cubic smoothing splines in the MESA sample. Age at baseline, sex, and the top 10 principal components (PCs) of the genetic relationship matrix were included as time-invariant covariates in the analysis. The top 10 PCs were calculated using the LD pruned SNPs with MAF > 0.05 to control for population structure. Since hypertension was assessed in year 2000, 2002, 2004, 2005, and 2010, we coded time at the five time points as 0, 0.2, 0.4, 0.5, and 1, respectively. We also applied the Gaussian copula method with heterogenous genetic effect, adjusting for the same covariates. To compare the performance of the varying-coefficient tests with the association tests assuming constant genetic effect, we applied RGMMAT and GMMAT to the analysis of hypertension, adjusting for age at baseline, sex, time, and the top 10 PCs.

The two retrospective tests, RVMMAT and RGMMAT, showed no evidence of inflation in the quantile-quantile (Q-Q) plot. The genomic control inflation factors were 0.905 and 0.976, respectively. The prospective VMMAT test was overly conservative, with a genomic control factor of 0.774, consistent with the observed deflation in the type I error simulations. The genomic control inflation factor was 0.838 for GMMAT.

None of the SNPs reached genome-wide significance at the p-value threshold of 5×10^{-8} that is widely used in GWAS. We adopted a less stringent p-value threshold of 5×10^{-7} to increase true positive discovery recommended by (Chen et al. (2021)). Table 2 reports the top SNPs for which at least one of the tests gives a p-value $< 5 \times 10^{-7}$. The smallest p-values of these eight SNPs were mostly generated by RVMMAT, except at the last two SNPs. VMMAT generated much larger p-values than RVMMAT, due to its conservativeness, while RGMMAT and GMMAT had comparable results. The Gaussian copula method produced p-values comparable to VMMAT, except at the last two SNPs. A cluster of six SNPs in LD ($r^2 > 0.97$), rs145659245, rs58265184, rs57719815, rs60197637, rs61327798, and rs142890225, located at 4p15, showed time-varying genetic effect on hypertension by RVMMAT (p-value = $6.78 \times 10^{-8} - 2.85 \times 10^{-7}$). Figure 2A demonstrated the estimated genetic effect over time at these SNPs where the estimated effect at each time point was obtained by using the observed trait values at that time point only. A straight line was used to connect the estimated values at two adjacent time points. We observed an increasing and then decreasing trend in genetic effects on hypertension across the five time points. However, RGMMAT and GMMAT lost power and generated large p-values by assuming constant genetic effect. These SNPs are in an intron of the gene *PROM1*, encoding a pentaspan transmembrane glycoprotein, which was reported to be associated with pulse pressure (Evangelou et al. (2018)). The smallest p-value for rs374012917, located on chromosome 17, was generated by the Gaussian copula method (p-value = 1.02×10^{-7}). As the estimated genetic effects at the five time points were relatively stable for this SNP (Figure 2B), RGMMAT and GMMAT generated slightly larger p-values. There was also evidence of association between hypertension and rs72930733 (p-value = 2.55×10^{-7}). Although RVMMAT did not give the smallest p-value for this SNP, its p-value was slightly larger than that of RGMMAT, mostly due to the increasing trend in genetic effect (Figure 2B). This SNP is in an intron of the gene *WDR7*, located at 18q21. Two hypertension GWAS identified an association between *WDR7* and systolic blood pressure (Evangelou et al. (2018), Kichaev et al. (2019)).

We further assessed the model fitting of cubic smoothing splines on the top SNPs in Table 2 using deviance and goodness-of-fit p-value. We calculated the deviance $D = -2 \sum_{i=1}^n \sum_{j=1}^{m_i} \{Y_{i,j} \ln(\frac{\hat{\mu}_{i,j}}{Y_{i,j}}) + (1 - Y_{i,j}) \ln(\frac{1 - \hat{\mu}_{i,j}}{1 - Y_{i,j}})\}$ of the working GLMM (5), as the goodness-of-fit test statistic, and compared it with a χ^2 distribution with degrees of freedom equal to the total number of observations minus the number of parameters in Model (5). All p-values were large, suggesting that there was no evidence of lack of fit (Table 3). We also checked the deviance residuals of the cubic smoothing splines model applied to the top SNPs. The deviance residuals range from -2.46 to 2.19 , suggesting that cubic smoothing splines fit the data adequately.

4.2. Pathway analysis. We then performed functional pathway analysis using the MetaCore™ software to identify enriched pathways related to hypertension. The top SNPs for which at least one of the tests had a p-value $< 2 \times 10^{-4}$ were included in the analysis. Fisher's exact test was used to determine whether the SNP list was enriched for a functional pathway. At the false discovery rate (FDR) < 0.05 , we identified two significant pathways that were associated with G-protein signaling and DNA damage. The first one is the G-protein signaling pathway related to Rac1 activation (p-value = 6.12×10^{-5} , FDR = 1.65×10^{-2}). Rac1 participates in the control of blood pressure through multiple mechanisms in the arterial wall and the central nervous system (Loirand and Pacaud (2010)). Importantly, a role for Rac1 in atherosclerosis and cardiac hypertrophy has been established in response to the administration of statins in clinical trials (Maack et al. (2003)). Animal studies indicated that Rac1 is essential for endothelium-dependent vasomotor response, the redox state of

TABLE 2
SNPs with p -value $< 5 \times 10^{-7}$ in at least one of the tests in the MESA data

Chr	Gene Region	SNP	Position	MAF	RVMMAT	VMMAT	Copula	RGMMAT	GMMAT
4	<i>PROM1</i>	rs145659245	16,060,553	0.013	6.78×10^{-8}	4.19×10^{-6}	9.77×10^{-6}	7.67×10^{-4}	1.78×10^{-3}
		rs58265184	16,061,151	0.014	2.18×10^{-7}	7.70×10^{-5}	2.79×10^{-5}	2.51×10^{-3}	4.85×10^{-3}
		rs57719815	16,063,652	0.013	2.85×10^{-7}	8.04×10^{-5}	3.09×10^{-5}	1.65×10^{-3}	3.27×10^{-3}
		rs60197637	16,063,659	0.013	2.85×10^{-7}	8.04×10^{-5}	3.09×10^{-5}	1.65×10^{-3}	3.27×10^{-3}
		rs61327798	16,063,661	0.013	2.85×10^{-7}	8.04×10^{-5}	3.09×10^{-5}	1.65×10^{-3}	3.27×10^{-3}
		rs142890225	16,065,544	0.013	2.85×10^{-7}	8.04×10^{-5}	3.10×10^{-5}	1.65×10^{-3}	3.27×10^{-3}
17	<i>LRRC37B</i>	rs374012917	30,403,054	0.038	2.21×10^{-5}	1.95×10^{-4}	1.02×10^{-7}	2.31×10^{-6}	4.95×10^{-6}
18	<i>WDR7</i>	rs72930733	54,641,870	0.011	1.80×10^{-6}	7.06×10^{-5}	7.72×10^{-6}	2.55×10^{-7}	4.63×10^{-6}

The smallest p -values among the five tests at the given SNPs are in bold.

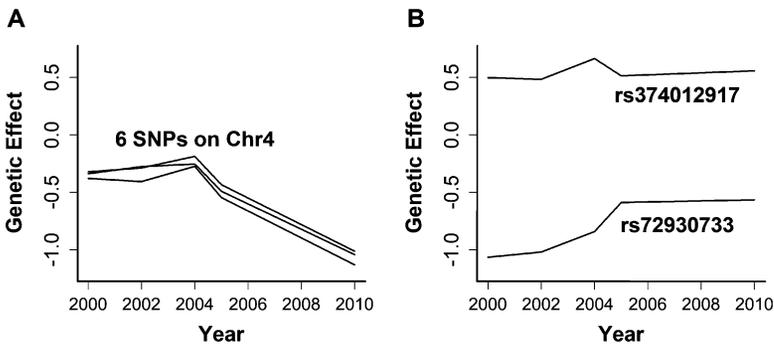


FIG. 2. Estimated genetic effect of the top eight SNPs on hypertension at each of the five time points: (A) six SNPs on chromosome 4, (B) two SNPs on chromosomes 17 and 18, respectively.

blood vessels, and homeostasis of blood pressure (Moustafa-Bayoumi et al. (2003), Satoh et al. (2006), Sawada et al. (2008)). The second pathway is the DNA damage pathway related to the ataxia-telangiectasia mutated (ATM) kinase activation ($p\text{-value} = 4.98 \times 10^{-4}$, $FDR = 4.48 \times 10^{-2}$). Emerging evidence has demonstrated that accumulated DNA damage and subsequent repair pathways play a crucial role in the initiation and progression of cardiovascular disorders, such as atherosclerosis and maladaptive cardiac hypertrophy (Shah et al. (2018), Shah and Mahmoudi (2015), Uryga, Gray and Bennett (2016), Wu et al. (2022)). ATM-mediated phosphorylation plays cardinal roles in response to genomic stress to preserve cellular homeostasis. DNA double-strand breaks trigger ATM activation which mediates DNA damage response and regulate cardiac remodeling, inflammation, and systolic function, eventually promoting heart failure development (Shiloh and Ziv (2013), Uziel et al. (2003)).

5. Discussion. In genome-wide association analysis of longitudinal traits, modeling time-varying genetic effect can increase power for the detection of genes underlying the development and progression of complex diseases. In this study we developed RVMMAT, a GLMM-based, retrospective varying-coefficient association testing method for longitudinal binary traits. RVMMAT extends the existing association methods, assuming constant effect over time to testing of time-varying effect on binary traits. RVMMAT is constructed based on the trait model, allowing for time-varying genetic effect. The variance of the test statistics is assessed retrospectively by considering the conditional distribution of the genotype at the variant of interest, given phenotype and covariate information, under the null hypothesis of no association. Our methodological contribution consists of designing joint tests using a

TABLE 3
Assessment of model fitting with cubic smoothing splines at the top SNPs in the MESA data

Chr	Gene Region	SNP	Position	MAF	Deviance	Goodness-of-fit P-value
4	<i>PROM1</i>	rs145659245	16,060,553	0.013	10,467.93	1
		rs58265184	16,061,151	0.014	10,468.13	1
		rs57719815	16,063,652	0.013	10,469.79	1
		rs60197637	16,063,659	0.013	10,469.79	1
		rs61327798	16,063,661	0.013	10,469.79	1
		rs142890225	16,065,544	0.013	10,469.79	1
17	<i>LRRC37B</i>	rs374012917	30,403,054	0.038	10,495.83	1
18	<i>WDR7</i>	rs72930733	54,641,870	0.011	10,493.05	1

Cauchy combination method for both prospective test and retrospective test for time-varying genetic effects on binary traits. The advantages of RVMMAT are: (1) it is computationally feasible for genetic studies with millions of variants, (2) it has well-controlled type I error in the presence of ascertainment and trait model misspecification, and (3) it can easily be fitted as a GLMM model using popular software such as R and SAS. We also propose VMMAT, a prospective varying-coefficient association test, for performance comparison.

Our simulation results demonstrated that RVMMAT maintained correct type I error under different trait models and ascertainment schemes, whereas VMMAT was overly conservative, due to the biased estimation of variance in the penalized trait model. We further demonstrated that the retrospective RVMMAT test achieved the highest power among the five tests under all the trait models and ascertainment schemes considered in the simulations. Application of RVMMAT to the MESA longitudinal hypertension data identified three novel genes that were associated with hypertension. Among them, two genes are known to be associated with systolic blood pressure and pulse pressure. Moreover, we identified two significant pathways associated with longitudinal hypertension: the G-protein signaling pathway related to Rac1 activation and the DNA damage pathway related to ATM activation. Given the established role for Rac1 and ATM in atherosclerosis and cardiac hypertrophy, our findings suggest that RVMMAT can provide enhanced statistical power in detecting biologically relevant genetic loci that are associated with trait dynamics. A better understanding of temporal variation of trait values and time-varying genetic contribution may shed light on the genetic mechanisms influencing the temporal trend of diseases and complex traits.

In many applications we are also interested in testing whether the genetic effect of a SNP is constant over time or time-varying. The prospective version of this test is the same as the hypothesis test on $\beta_1(t)$ developed in Section 4 of (Zhang (2004)). In retrospective analysis it is more challenging because the genotype model under the null hypothesis of constant genetic effect is difficult to specify, especially for the covariance of genotypes at the SNP when it is associated with the trait. The genetic relationship matrix represents the overall genetic similarity between samples and does not reflect the correlation of genotypes at that SNP, that is, the null conditional mean and conditional covariance of the genotype vector in equation (10) do not hold anymore. We are currently exploring a retrospective test of whether the genetic effect is constant vs. time-varying and will report it in our future work.

The RVMMAT and VMMAT methods are designed for single-variant association analysis of longitudinal binary traits. However, single-variant association tests suffer from restricted power to detect association for rare variants in whole-genome sequencing studies. As many variants influence complex traits collectively, assessing joint effects from multiple variants by aggregating weak signals at the gene or pathway level holds great promise for the identification of novel genes underlying disease risks. To extend RVMMAT to rare variant analysis with longitudinal binary data, we could design a linear statistic or a quadratic statistic that combines the test allowing for time-varying genetic effect at each variant in a gene region. Such statistics are likely to better calibrate the fluctuation of genetic contributions to the trait values over time. Additionally, our current model can easily be extended to analyze nominal, ordinal and count data.

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SUPPLEMENTARY MATERIAL

Supplement A: Algorithm for fitting GLMM (DOI: [10.1214/23-AOAS1798SUPPA](https://doi.org/10.1214/23-AOAS1798SUPPA); .pdf). Detail of the algorithm to estimate the fixed effects and the random effects in the GLMM (5).

Supplement B: Computer code (DOI: [10.1214/23-AOAS1798SUPPB](https://doi.org/10.1214/23-AOAS1798SUPPB); .zip). R package implementing RVMAT and VMMAT and simulation code.

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