Received: 13 June 2018 | Revised: 27 August 2018 | Accepted: 26 September 2018

DOI: 10.1002/gepi.22177

RESEARCH ARTICLE

WILEY Genetic Epidemiology

OFFICIAL JOURNAL INTERNATIONAL GENETIC EPIDEMIOLOGY SOCIETY www.geneticepi.org

Linear mixed models for association analysis of quantitative traits with next-generation sequencing data

Chi-yang Chiu[1,2] | Fang Yuan[3] | Bingsong Zhang[4] | Ao Yuan[4] | Xin Li[4] | Hong-Bin Fang[4] | Kenneth Lange[5] | Daniel E. Weeks[6,7] | Alexander F. Wilson[2] | Joan E. Bailey-Wilson[2] | Anthony M. Musolf[2] | Dwight Stambolian[8] | M'Hamed Lajmi Lakhal-Chaieb[9] | Richard J. Cook[10] | Francis J. McMahon[11] | Christopher I. Amos[12] | Momiao Xiong[13] | Ruzong Fan[2,4]

[1]Division of Biostatistics, Department of Preventive Medicine, University of Tennessee Health Science Center, Memphis, Tennessee

[2]Computational and Statistical Genomics Branch, National Human Genome Research Institute, National Institutes of Health (NIH), Bethesda, Maryland

[3]Department of Biochemistry and Molecular Biology, School of Basic Medicine, Kunming Medical University, Kunming, Yunnan, China

[4]Department of Biostatistics, Bioinformatics, and Biomathematics, Georgetown University Medical Center, Washington, District of Columbia

[5]Department of Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, California

[6]Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

[7]Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

[8]Department of Genetics, University of Pennsylvania, Philadelphia, Pennsylvania

[9]Department de Mathematiques et de Statistique, Universite Laval, Quebec, Canada

[10]Department of Statistics and Actuarial Science, University of Waterloo, Waterloo, Ontario, Quebec, Canada

[11]Human Genetics Branch and Genetic Basis of Mood and Anxiety Disorders Section, National Institute of Mental Health, NIH, Bethesda, Maryland

[12]Department of Medicine, Baylor College of Medicine, Houston, Texas

[13]Human Genetics Center, University of Texas-Houston, Houston, Texas

Correspondence
Ruzong Fan, Department of Biostatistics, Bioinformatics, and Biomathematics, Georgetown University Medical Center, 4000 Reservoir Road NW, Building D-180, Washington, DC 20057.
Email: rf740@georgetown.edu

Funding information
National Human Genome Research Institute; National Institute of Mental Health; National Institutes of Health; NIH, Grant/Award Number: R01EY024226; Yunnan Applied Basic Research Projects China, Yunnan Province, P. R. China, Grant/Award Number: U0120170557

Abstract

We develop linear mixed models (LMMs) and functional linear mixed models (FLMMs) for gene-based tests of association between a quantitative trait and genetic variants on pedigrees. The effects of a major gene are modeled as a fixed effect, the contributions of polygenes are modeled as a random effect, and the correlations of pedigree members are modeled via inbreeding/kinship coefficients. F-statistics and χ^2 likelihood ratio test (LRT) statistics based on the LMMs and FLMMs are constructed to test for association. We show empirically that the F-distributed statistics provide a good control of the type I error rate. The F-test statistics of the LMMs have similar or higher power than the FLMMs, kernel-based famSKAT (family-based sequence kernel association test), and burden test famBT (family-based burden test). The F-statistics of the FLMMs perform well when analyzing a combination of rare and common variants. For small samples, the LRT statistics of the FLMMs control the type I error rate well at the nominal levels

Chi-yang Chiu and Fang Yuan contributed equally to this work.

$\alpha = 0.01$ and 0.05. For moderate/large samples, the LRT statistics of the FLMMs control the type I error rates well. The LRT statistics of the LMMs can lead to inflated type I error rates. The proposed models are useful in whole genome and whole exome association studies of complex traits.

KEYWORDS

common variants, complex diseases, functional data analysis, functional linear mixed models, linear mixed models, rare variants

1 | INTRODUCTION

Next-generation sequencing allows nearly complete evaluation of genetic variation, including several million common (e.g., $\geq 1\%$ population frequency) and rare variants (e.g., $< 1\%$ population frequency) (Abecasis et al., 2012; Lek et al., 2016; Rusk & Kiermer, 2008; Tennessen et al., 2012). Thus, it is important to properly reduce the high dimensionality of next-generation sequencing data to draw useful information. Rare variants have very low frequencies, so the power of single variant-by-variant association analysis of rare variants is limited. It is, therefore, necessary to group the rare variants to perform gene-based analysis. In recent years, there has been great interest in developing statistical methods to analyze rare variants using grouped region-based tests.

The existing gene-based analysis methods fall into three classes: (a) burden tests (BTs), (b) kernel tests, and (c) fixed effect regression models. BTs are based on collapsing rare variants to a single variable, which is then used to test for an association with the phenotypes (Han & Pan, 2010; Li & Leal, 2008; Morris & Zeggini, 2010; Price et al., 2010). The kernel-based tests, such as sequence kernel association test (SKAT), its optimal unified test (SKAT-O), a combined sum test of rare and common variant effects (SKAT-C), and family-based SKAT (famSKAT), all aggregate the association between variants and phenotypes via a kernel matrix, which measures the similarity between individuals (Chen, Meigs, & Dupuis, 2013; Ionita-Laza, Lee, Makarov, Buxbaum, & Lin, 2013; Lee et al., 2012; Wu et al., 2011). It was found that SKAT/SKAT-O tests have higher power than BTs, such as the combined collapsing and multivariate method, nonparametric weighted sum test (Madsen & Browning, 2009), and the cohort allelic sums test (Morgenthaler & Thilly, 2007).

Fixed regression models can be either traditional additive models or functional regression models. By using functional data analysis (fda) techniques, a class of fixed effect functional models is developed to test associations between complex traits (i.e., a quantitative or binary or survival trait) and genetic variants for unrelated population samples adjusting for covariates (Fan et al., 2013, 2014; Fan, Wang, et al., 2016; Luo, Boerwinkle, & Xiong, 2011; Luo, Zhu, & Xiong, 2012, 2013; Vsevolozhskaya, Zaykin, Greenwood, Wei, & Lu, 2014; Vsevolozhskaya et al., 2016). The functional models are very flexible and can analyze rare variants or common variants or a combination of the two. The basic idea of functional regression models is to treat multiple genetic variants of an individual in a human population as a realization of an underlying stochastic process (Ross, 1996). The genome of an individual is viewed as a stochastic function, which contains both physical position and linkage disequilibrium (LD) information of the genetic markers. In these models, the genome of an individual in a chromosome region is treated as a continuum of sequence data rather than discrete observations.

For unrelated samples, functional regression-based statistics have been built to test for association between phenotypic traits and genetic variants. Extensive simulations and real data analysis demonstrate that the fixed effect functional models perform better in major gene analysis than SKAT/SKAT-O/SKAT-C (Fan, Chiu, et al., 2016). To date, the functional regression models have only been developed to analyze unrelated population data except for the famFLM method (Svishcheva, Belonogova, & Axenovich, 2015). As members of a pedigree are correlated with each other, existing functional regression models cannot be directly applied to familial data. There is a need to extend the models to analyze extended pedigrees, properly taking pedigree member relatedness into account (Jiang et al., 2018).

Here, we consider additive linear mixed models (LMMs), which are widely used for quantitative trait association studies because they have two remarkable features. First, LMMs accurately control the type I error rates and properly correct for confounding arising from population stratification, family structure, and cryptic relatedness. Second, LMMs can be applied to samples with arbitrary combinations of related and unrelated individuals. However, LMMs so far are mainly designed for testing the association of common variants with quantitative traits

(Astle & Balding, 2009; Aulchenko, de Koning, & Haley, 2007; Kang et al., 2010; Korte et al., 2012; Lippert et al., 2011; Listgarten et al., 2012; Yang, Zaitlen, Goddard, Visscher, & Price, 2014; Yu et al., 2006; Zhou & Stephens, 2012). They are typically carried out by testing the association of a single variant, one at a time. There has been very little research on how to utilize LMMs to perform gene-based analysis of rare variants or a combination of rare and common variants to analyze extended families.

To take advantage of both LMMs and fda simultaneously, we build functional linear mixed models (FLMMs) to connect the phenotypic traits to the genetic variants. Our motivation arises from the superior performance of functional regression models in analyzing unrelated data and the expectation that this advantage should carry over to the analysis of pedigree data. As in the functional regression models developed previously, the effect of genetic variants is modeled by a genetic effect function. The contribution of polygenes is modeled as a separate random variation, and the correlation of pedigree members is taken care of by kinship coefficients. The LMMs and FLMMs are an extension of traditional variance component models (Amos, 1994; Lange, 2002). We evaluate the performance of the LMMs and FLMMs via extensive simulations and illustrate their application by analyzing a complex trait, refractive error, with exome chip genotyping of Amish pedigrees (Musolf et al., 2017, 2018; Wojciechowski, Bailey-Wilson, & Stambolian, 2009; Wojciechowski, Stambolian et al., 2009).

2 | METHODS

Consider a sample consisting of multiple families. To simplify notation, we consider one pedigree with n individuals labeled $i = 1, 2, ..., n$; each individual i is preceded by all of his/her ancestors. We denote the quantitative traits of the pedigree members by a trait vector $\mathbf{Y} = (y_1, y_2, ..., y_n)'$, where $'$ denotes the transpose. All individuals in each family are sequenced in a genomic region that has m genetic variants with ordered genetic locations $0 \le t_1 < \cdots < t_m = T$. Here, we assume that the base pair position t_ℓ of each variant is known. We normalize the region $[t_1, T]$ to be $[0, 1]$. For the ith individual, let $X_i = (x_i(t_1), ..., x_i(t_m))'$ denote the genotypes, coded as the number of minor alleles at each of the m variants, and $Z_i = (z_{i1}, ..., z_{ic})'$ denote the covariates.

For the n individuals who are phenotyped and sequenced, let Ω be an $n \times n$ matrix containing diagonal elements $\Omega_{ii} = 1 + h_i$, where h_i is the inbreeding coefficient for individual i, and off-diagonal elements $\Omega_{ij} = 2\phi_{ij}$. The parameter ϕ_{ij} is the kinship coefficient between individuals i and j, the probability that a randomly chosen allele at a given locus from individual i is identical by descent to a randomly chosen allele from individual j conditional on their ancestral relationship.

2.1 | Linear mixed effect models

2.1.1 | LMMs

Here, we assume that the trait vector $\mathbf{Y} = (y_1, y_2, ..., y_n)'$ follows a multivariate normal distribution. By using genotype data directly, we may relate the genetic variants to the trait adjusting for covariates by the following additive LMM:

$$y_i = \alpha_0 + Z_i'\alpha + \sum_{\ell=1}^{m} x_i(t_\ell)\beta_\ell + G_i + e_i, \qquad (1)$$

where α_0 is an overall mean, α is a $c \times 1$ vector of fixed regression coefficients of the covariates, β_ℓ is the effect of the genetic variant $x_i(t_\ell)$, $(G_1, ..., G_n)'$ is a normal random vector with mean 0 and covariance matrix $\sigma_G^2\Omega$, and $(e_1, ..., e_n)'$ is a normal vector of error terms with $N(0, \sigma_e^2 I_{n\times n})$. Here, σ_G^2 is a polygeneic variance component, G_i is an additive polygenic variation, and $I_{n\times n}$ is an identity matrix. We assume that G_i and e_i are independent. Before fitting the LMM (1), QR decomposition can be applied to the genotype data to decompose the genotype matrix into the product of an orthogonal matrix Q and a triangular matrix R via the Gram–Schmidt process.

2.1.2 | General FLMMs

We denote the genetic variant function (GVF) of the ith individual by $X_i(t)$, $t \in [0, 1]$. By using the genetic information X_i, we may estimate the related GVF $X_i(t)$. To model the relationship between the trait and the GVF $X_i(t)$, consider the following FLMM:

$$y_i = \alpha_0 + Z_i'\alpha + \int_0^1 X_i(t)\beta(t)\,dt + G_i + e_i, \qquad (2)$$

where $\beta(t)$ is the genetic effect of GVF $X_i(t)$ at the location t and the other terms are the same as additive LMM (1). In the FLMM (2), the GVF $X_i(t)$ and genetic effect function $\beta(t)\,dt$ are assumed to be continuous. The continuity of the GVF $X_i(t)$ can be relaxed by considering a model, where $\beta(t)$ is a smooth function, see below.

2.1.3 | Beta-smooth only FLMMs

To remove the assumption of the continuity of the GVF $X_i(t)$ in the FLMM (2), a simplified functional LMM is obtained by replacing the integration term $\int_0^1 X_i(t)\beta(t)\,dt$ in Model (2) by the summation term $\sum_{\ell=1}^{m} x_i(t_\ell)\beta(t_\ell)$. That is, we have

$$y_i = \alpha_0 + Z_i'\alpha + \sum_{\ell=1}^{m} x_i(t_\ell)\beta(t_\ell) + G_i + e_i, \quad (3)$$

where $\beta(t_\ell)$ is the genetic effect at the location t_ℓ and the other terms are similar to those in the additive LMM (1) and the general FLMM (2). In our previous study, we show that a beta-smooth only model performs very similarly to the general functional linear models in applications and simulation studies for population data, in which the G_i term is not included (Fan et al., 2013, 2014; Fan, Wang, et al., 2016).

2.2 | Revised FLMMs

2.2.1 | Expansion of the genetic effect function

The genetic effect function $\beta(t)$ in Models (2) and (3) is assumed to be smooth. One may expand it using B-spline or Fourier basis functions. We expand the genetic effect function $\beta(t)$ using a series of K_β basis functions $\psi_1(t),...,\psi_{K_\beta}(t)$ as $\beta(t) = \psi(t)'\beta$, where $\beta = (\beta_1,...,\beta_{K_\beta})'$ is a vector of coefficients $\beta_1,...,\beta_{K_\beta}$ and $\psi(t) = (\psi_1(t),...,\psi_{K_\beta}(t))'$. We consider two types of basis functions: (a) The B-spline basis function, where $\psi_k(t) = B_k(t)$, $k = 1,...,K_\beta$ and (b) the Fourier basis function, where $\psi_1(t) = 1$, $\psi_{2r+1}(t) = \sin(2\pi rt)$, and $\psi_{2r}(t) = \cos(2\pi rt)$, $r = 1,...,(K_\beta - 1)/2$. Here, for the Fourier basis, K_β is a positive odd integer (de Boor, 2001; Ferraty & Romain, 2010; Horváth & Kokoszka, 2012; Ramsay, Hooker, & Graves, 2009; Ramsay & Silverman, 2005).

2.2.2 | Estimation of the GVF

To estimate the GVFs $X_i(t)$ from the genotypes X_i, we use an ordinary linear square smoother. Let $\phi_k(t)$, $k = 1,...,K$ be a series of K basis functions, such as the B-spline basis and Fourier basis functions, and let $\phi(t) = (\phi_1(t),...,\phi_K(t))'$. Let Φ denote the $m \times K$ matrix containing the values $\phi_k(t_\ell)$. Using the discrete realizations $X_i = (x_i(t_1),...,x_i(t_m))'$, we may estimate the GVF $X_i(t)$ using an ordinary linear square smoother as follows:

$$\hat{X}_i(t) = (x_i(t_1),...,x_i(t_m))\Phi[\Phi'\Phi]^{-1}\phi(t). \quad (4)$$

2.2.3 | Revised FLMMs

Here, we expand $X_i(t)$ by the ordinary linear square smoother (4). We expand the genetic effect function $\beta(t)$ as $\beta(t) = (\psi_1(t),...,\psi_{K_\beta}(t))(\beta_1,...,\beta_{K_\beta})' = \psi(t)'\beta$. Replacing $X_i(t)$ in the FLMM (2) by $\hat{X}_i(t)$ in (4) and $\beta(t)$ by the expansion $\psi(t)'\beta$, we have a revised LMM:

$$\begin{aligned} y_i &= \alpha_0 + Z_i'\alpha \\ &\quad + [(x_i(t_1),...,x_i(t_m))\Phi[\Phi'\Phi]^{-1}\int_0^1 \phi(t)\psi'(t)\,dt]\beta \\ &\quad + G_i + e_i \\ &= \alpha_0 + Z'_i\alpha + W'_i\beta + G_i + e_i, \end{aligned} \quad (5)$$

where $W_i' = (x_i(t_1),...,x_i(t_m))\Phi[\Phi'\Phi]^{-1}\int_0^1 \phi(t)\psi'(t)\,dt$. In the above-revised Model (5), one needs to calculate $\Phi[\Phi'\Phi]^{-1}$ and $\int_0^1 \phi(t)\psi'(t)\,dt$ to get W_i. In the statistical R package (The R Project for Statistical Computing, https://www.r-project.org/) *fda* or *Matlab*, code is readily available to calculate them (Ramsay et al., 2009).

2.2.4 | Revised beta-smooth only FLMMs

In Model (3), $\beta(t_\ell)$ is introduced as the genetic effect at the location t_ℓ. We assume that the genetic effect function $\beta(t)$ is a function of the genetic location t. Therefore, $\beta(t_\ell)$, $\ell = 1, 2, ..., m$, are the values of function $\beta(t)$ at the m genetic locations. The genetic effect function $\beta(t)$ is assumed to be smooth. One may expand it by B-spline or Fourier or linear spline basis functions as above. Replacing $\beta(t_\ell)$ by the expansion, Model (3) can be revised as

$$\begin{aligned} y_i &= \alpha_0 + Z_i'\alpha \\ &\quad + \left[\sum_{\ell=1}^{m} x_i(t_\ell)(\psi_1(t_\ell),...,\psi_{K_\beta}(t_\ell))\right](\beta_1,...,\beta_{K_\beta})' \\ &\quad + G_i + e_i \\ &= \alpha_0 + Z'_i\alpha + W'_i\beta + G_i + e_i, \end{aligned} \quad (6)$$

where $W_i' = \sum_{\ell=1}^{m} x_i(t_\ell)(\psi_1(t_\ell),...,\psi_{K_\beta}(t_\ell))$. In Model (3) and its revised version (6), we use the raw genotype data $X_i = (x_i(t_1),...,x_i(t_m))'$ directly in the analysis. In addition, we assume that the genetic effect function $\beta(t)$ is smooth. Hence, we call the models the "beta-smooth only" approach.

2.3 | Dealing with missing genotype data

If some genotype data are missing, the FLMMs can be modified to analyze the data. For example, assume there is no genotype information at the first variant for the ith individual (i.e., we only have $X_i = (?, x_i(t_2),...,x_i(t_m))'$). Let Φ_1 denote the $(m-1) \times K$ matrix containing the values $\phi_k(t_j)$, where $j \in 2, ..., m$. Then, we may revise the estimate (4) as

$$\hat{X}_{\ell i}(t) = \phi(t)'[\Phi'_1\Phi_1]^{-1}\Phi'_1(x_i(t_2),...,x_i(t_m))'. \quad (7)$$

Note that the estimate (7) only depends on the available genotype data $(x_i(t_2),...,x_i(t_m))'$. Hence, each individual's

GVF is estimated by his/her own nonmissing data, a practical advantage of the fda approach. Using (7), one may revise the FLMM (2) to be a form of Model (5) accordingly.

If, for example, $X_i = (?, x_i(t_2), ..., x_i(t_m))'$, where $x_i(t_1)$ is missing, we may revise the beta-smooth only FLMM (3) as

$$y_i = \alpha_0 + Z'_i\alpha + \sum_{\ell=2}^{m} x_i(t_\ell)\beta(t_\ell) + G_i + e_i. \quad (8)$$

The revised FLMM (8) only depends on the available genotype data $(x_i(t_2), ..., x_i(t_m))'$, and it can be revised accordingly to be a form of Model (6) as

$$y_i = \alpha_0 + Z'_i\alpha + \left[\sum_{\ell=2}^{m} x_i(t_\ell)(\psi_1(t_\ell), ..., \psi_{K_\beta}(t_\ell))\right](\beta_1, ..., \beta_{K_\beta})' + G_i + e_i.$$

2.4 | Likelihood of LMMs and FLMMs

The log-likelihood is defined by

$$L = -\frac{n}{2}\log(2\pi) - \frac{1}{2}\log|\Sigma| - \frac{1}{2}(\mathbf{Y} - E\mathbf{Y})'\Sigma^{-1}(\mathbf{Y} - E\mathbf{Y}).$$

In the log-likelihood L, the mean component $E\mathbf{Y}$ is $E(y_i) = \alpha_0 + Z_i'\alpha + \sum_{\ell=1}^{m} x_i(t_\ell)\beta_\ell$ for the additive LMM (1) and $\alpha_0 + Z_i'\alpha + W_i'\beta$ for the FLMMs (5) and (6), and Σ is an $n \times n$ variance–covariance matrix defined as $\Sigma = \sigma_G^2\Omega + \sigma_e^2 I_{n\times n}$. Note that typically the variance–covariance matrix differs from pedigree to pedigree. Under the normality assumption of the LMM, the marginal likelihood has a closed form and maximum likelihood estimation can be performed conveniently for quantitative traits.

2.5 | Parameter estimation and test statistics

To test for association between the quantitative trait and the genetic variants, the null hypothesis is H_0: $\beta = 0$. Under the null hypothesis, the FLMMs (5) and (6) simplify to

$$y_i = \alpha_0 + Z_i'\alpha + G_i + e_i. \quad (9)$$

The null LMM (9) is also a null model of LMM (1). The LMM (1) or FLMM (5) or (6) and the null Model (9) are nested. To facilitate parameter estimation, we use the Cholesky decomposition of the covariance structure. Briefly, let $\Sigma = LL'$, where L is the Cholesky factor. Let us denote $\mathbf{X} = L^{-1}\mathbf{Y}$. Then, we have $\mathrm{Var}(\mathbf{X}) = L^{-1}\Sigma(L')^{-1} = I_n$. Therefore, the transformed traits $\mathbf{X}$ are standard normal and can be analyzed as independent data. By using the transformed traits $\mathbf{X}$, we may reformulate the null Model (9) as

$$\mathbf{X} = L^{-1}Z\begin{pmatrix}\alpha_0\\ \alpha\end{pmatrix} + \boldsymbol{\varepsilon}, \quad (10)$$

where

$$Z = \begin{pmatrix}1 & Z'_1\\ \vdots & \vdots\\ 1 & Z'_n\end{pmatrix}$$

and $\boldsymbol{\varepsilon}$ is a vector of independent standard normal variables. Similarly, the FLMM (5) or (6) can be rewritten as

$$\mathbf{X} = L^{-1}Z\begin{pmatrix}\alpha_0\\ \alpha\end{pmatrix} + L^{-1}W'\beta + \boldsymbol{\varepsilon}, \quad (11)$$

where $W = (W_1, ..., W_n)$. The LMM (1) can be rewritten using $\mathbf{X}$ as a form of Model (11). By fitting Models (10) and (11), we may test the null $H_0: \beta = 0$ using an F-distributed or a χ^2-distributed likelihood ratio test (LRT) statistic.

2.6 | Simulation studies

To evaluate the performance of the test statistics, we simulated data to estimate empirical type I error rates and power levels. In our simulations, we consider a variant to be rare if its minor allele frequency (MAF) is <0.03. Two scenarios were considered: (a) Some variants are common and the rest are rare and (b) all variants are rare.

2.6.1 | Pedigree template A of 25 families

We first simulated 25 families by randomly choosing progeny sizes from a negative binomial distribution. We assumed that each child within the second generation has a 25% chance of having offspring. The final structure of the pedigrees included 228 individuals (119 males and 109 females; 70 founders and 158 nonfounders). The pedigree size ranged from 4 to 24 with an average value of 9.12.

2.6.2 | Pedigree template B of 50 families

By doubling the 25 families, the pedigree structures included 456 individuals (238 males and 218 females; 140 founders and 316 nonfounders) within 50 families.

2.6.3 | Pedigree template C of 75 families

By tripling the 25 families, the pedigree structures included 684 individuals (357 males and 327 females; 210 founders and 474 nonfounders) within 75 families.

2.6.4 | Genetic variants

The sequence data are of European ancestry from 10,000 chromosomes covering a 1-Mb region, simulated by Yun Li at the University of North Carolina, Chapel Hill using the calibrated coalescent model as programmed in COSI (Schaffner et al., 2005, available at: http://www.broadinstitute.org/~sfs/). The sequence data were generated using COSI's calibrated best-fit models, and the generated European haplotypes mimic centre d'Etude du polymorphisme humain (CEPH) Utah individuals with ancestry from northern and western Europe in terms of site frequency spectrum and LD patterns (The International HapMap Consortium, 2007). To evaluate empirical type I error rates and power, we used a gene-dropping simulation approach, first randomly sampling two haplotypes for each founder. Then, for each nonfounder in the pedigree, we chose one haplotype at random from each of his or her parents. Genotypes were constructed by summing up two haplotypes for each individual to determine the number of minor alleles at each base pair (bp) position within the 1-Mb region, assuming no recombination events in this small region during meioses.

2.6.5 | Type I error simulations

To evaluate type I error rates of the F-test and LRT statistics, we utilized the three pedigree templates A, B, and C described above. For each pedigree template set, we generated phenotype data sets using the model

$$y_i = \alpha_0 + z_{i1} + z_{i2} + G_i + e_i, \tag{12}$$

where $\alpha_0 = -4.60$, z_{i1} is a dichotomous covariate taking on values 0 and 1 with a probability of 0.5, z_{i2} is a continuous covariate from a standard normal distribution $N(0, 1)$, $\sigma_G = 0.2$, $\sigma_e = 0.75$, and $(G_1, ..., G_n)'$ is generated as a normal vector with mean 0 and a covariance matrix $\sigma_G^2\Omega$.

Genotypes were selected from variants in 3,6,..., 27,30 kb subregions randomly selected from the 1-Mb region. Notice that the trait values are not related to the genotypes and so the null hypothesis holds. For each simulation scenario, 10^6 phenotype–genotype data sets were generated; for each data set, we fit the models and calculated the test statistics and related p-values. Then, an empirical type I error rate was calculated as the proportion of 10^6 p-values, which were smaller than a given α level.

2.6.6 | Empirical power simulations

To evaluate the power of the F-test and LRT statistics, trait values were generated for each individual based upon the genotypes. To do this, we considered an LMM. We simulated data sets under the alternative hypothesis by randomly selecting subregions to obtain causal variants. First, we generated genotypes of m variants in a selected subregion, similar to the type I error simulations. Then, M of the m variants were randomly selected to be causal, yielding causal genotypes $(x_i(u_1), ..., x_i(u_M))$. For each data set, the causal variants are the same for all the individuals in the data set, but we allow the causal variants to be different from data set to data set. Then, we generated the quantitative traits by

$$y_i = \alpha_0 + z_{i1} + z_{i2} + \beta_1 x_i(t_1) + \cdots + \beta_M x_i(t_M) + G_i + e_i, \tag{13}$$

where α_0, z_{i1}, z_{i2}, and $(G_1, ..., G_n)'$ were the same as in the type I error Model (12), $(x_i(t_1), ..., x_i(t_M))'$ were genotypes of the ith individual at the causal variants, and the β's are additive effects for the causal variants defined as follows. In Model (13), we used $|\beta_j| = c|\log_{10}(\text{MAF}_j)|/2$, where c is defined below and MAF_j is the MAF of the jth variant. Three different settings were considered: 5%, 10%, and 15% of variants in the subregions are chosen as causal variants. Here, for the scenario where some variants are common and the rest are rare, the percentage is over all variants; and for the scenario where all variants are rare, the percentage is over all rare variants. When 5%, 10%, and 15% of the variants were causal, $c = \log(30)/k$, $\log(20)/k$, and $\log(15)/k$, respectively. For the template C of 75 two- or three-generation families, k increases and genetic effect sizes decrease as region sizes increase:

$$k = \begin{cases} 1.00 & \text{if region size=3 kb,} \\ 1.25 & \text{if region size=6 kb,} \\ 1.50 & \text{if region size=9 kb,} \\ 1.75 & \text{if region size=12 kb,} \\ 2.00 & \text{if region size=15 kb,} \\ 2.25 & \text{if region size=18 kb,} \\ 2.50 & \text{if region size=21 kb} \\ 2.75 & \text{if region size=24 kb,} \\ 3.00 & \text{if region size=27 kb,} \\ 3.25 & \text{if region size=30 kb.} \end{cases} \tag{14}$$

In addition to varying the percentage of causal variants in the subregion, we also varied the direction of effect. We considered situations where (a) all causal variants have positive effects, (b) 20%/80% causal variants have negative/positive effects, and (c) 50%/50% causal variants have negative/positive effects. BTs are expected to be most powerful when all of the causal variants have effects in the same direction [e.g., under scenario (a)]. For each setting, 1,000 data sets were simulated to calculate the empirical power as the proportion of p-values, which are smaller than a given α level.

2.7 | Analysis of refractive error data in the Myopia Family Study

To evaluate the performance of the F-test and LRT statistics in a more realistic setting, we exploit exome chip genotypes and a quantitative trait, refractive error measured in Diopters, from Amish families that are part of the Myopia Family Study (Wojciechowski, Bailey-Wilson, et al., 2009; Wojciechowski, Stambolian, et al., 2009). After sample quality checks using a thorough and rigorous data cleaning pipeline, which included checks for chromosomal aberrations, gender, Hardy–Weinberg equilibrium, relatedness, duplicates, and genotype quality, 300 genotyped and phenotyped individuals were available for analysis (see Wojciechowski, Bailey-Wilson, et al., 2009, for details of quality control on phenotype data; Musolf et al., 2017, 2018, for details of exome chip genotype data quality control processes). To completely specify the pedigree structures, we included nongenotyped or nonphenotyped individuals who shared the same family with phenotyped and genotyped family members. The connected pedigrees contained 409 pedigree members who are used to calculate the kinship coefficients. A total number of 52,035 autosomal variants were included in the study within 8,282 genes, which contain at least two variants and 1,572 genes, which contain at least six variants. As refractive error is nonnormally distributed in this sample, inverse normal rank transformation was applied before association analysis. We adjusted for gender because it is significantly associated with refractive error in the null model ($p = 0.049$).

2.8 | fda parameters

In the data analysis and simulations described above, we used functions in the R package *fda* (Ramsay et al., 2009) to create the basis functions. In the simulations presented in the main text and Supporting Information **I**, the order of the B-spline basis was 4, the number of B-spline basis functions was $K = K_\beta = 20$, and the number of Fourier basis functions was $K = K_\beta = 21$. To make sure that the results are valid and stable, we examined a wide range of parameters: $6 \leq K = K_\beta \leq 27$ for the B-spline and Fourier basis functions.

As most genes contain only a few variants in the Myopia Family Study data, we took a conservative strategy when analyzing the data: The order of the B-spline basis was 4, the number of B-spline basis functions was $K = K_\beta = 6$, and the number of Fourier basis functions was $K = K_\beta = 7$.

3 | RESULTS

3.1 | Simulation results

In this section, we present simulation results for the type I error rates and power levels using bar plots, where the statistics evaluated in the figures are identified using the abbreviations defined in Table 1. In the table, five F-distributed statistics, famSKAT, and famBT are presented. The five F-distributed statistics are based on the additive LMM (1) and FLMMs (5) and (6). The famSKAT and famBT are from Chen et al. (2013) (see also Oualkacha et al., 2013; Schifano et al., 2012).

3.1.1 | Simulations investigating type I error rates

Extensive simulations were carried out, comparing the type I error rates at five nominal significance levels of the five different F-distributed statistics (listed in Table 1) and five LRT statistics, varying the region size from 3 to 30 kb. The empirical type I error rates are reported at five

TABLE 1 Abbreviations used in the main text and figures

Notation	Description and interpretation
LMMs	Linear mixed models
FLMMs	Functional linear mixed models
GVF	Genetic variant function
F_FLMM_BS	F-test of FLMM (5) with the B-spline basis vs. null model (9)
F_FLMM_FR	F-test of FLMM (5) with the Fourier basis vs. null model (9)
F_beta_BS	F-test of FLMM (6) with the B-spline basis vs. null model (9)
F_beta_FR	F-test of FLMM (6) with the Fourier basis vs. null model (9)
F_add_LMM	F-test of additive LMM (1) vs. null model (9)
famSKAT	Family-based sequence kernel association test
famBT	Family-based burden tests

TABLE 2 Empirical type I error rates of the F-distributed statistics and LRT statistics at nominal levels $\alpha = 0.05, 0.01, 0.001, 0.0001$, and 0.00001 using the 75 two- or three-generation families with a total of 684 individuals as a template, when region sizes are 3,6, ..., 27,30 kb and some variants are common and the rest are rare

		Type I error rates of F-distributed statistics					Type I error rates of LRT statistics				
	Nominal level	FLMM (5)		FLMM (6)		LMM	FLMM (5)		FLMM (6)		LMM
Region size (# variants)	α	B-spline	Fourier	B-spline	Fourier	(1)	B-spline	Fourier	B-spline	Fourier	(1)
3 kb	0.05	0.021025	0.048638	0.041797	0.047574	0.048790	0.022653	0.051593	0.044490	0.050484	0.051852
	0.01	0.003591	0.009408	0.007930	0.009209	0.009476	0.004156	0.010616	0.008997	0.010415	0.010763
(59)	0.001	0.000291	0.000885	0.000735	0.000852	0.000879	0.000382	0.001118	0.000903	0.001076	0.001120
	0.0001	2.50E−05	9.00E−05	6.50E−05	9.10E−05	8.40E−05	3.60E−05	0.000117	9.40E−05	0.000113	0.000114
	1.00E−05	2.00E−06	1.20E−05	8.00E−06	1.20E−05	1.00E−05	5.00E−06	1.70E−05	1.30E−05	1.60E−05	1.50E−05
6 kb	0.05	0.044983	0.047848	0.047674	0.047708	0.047241	0.048454	0.051794	0.051317	0.051651	0.052409
	0.01	0.008377	0.008924	0.008965	0.008946	0.008757	0.009705	0.010420	0.010412	0.010467	0.010730
(117)	0.001	0.000747	0.000799	0.000809	0.000794	0.000832	0.001008	0.001095	0.001080	0.001082	0.001182
	0.0001	7.20E−05	7.20E−05	7.80E−05	7.10E−05	7.00E−05	0.000109	0.000123	0.000114	0.000123	0.000134
	1.00E−05	7.00E−06	5.00E−06	7.00E−06	5.00E−06	1.10E−05	1.60E−05	1.10E−05	1.50E−05	1.10E−05	1.90E−05
9 kb	0.05	0.047737	0.047766	0.047946	0.047773	0.046838	0.051529	0.051828	0.051733	0.051831	0.053811
	0.01	0.009185	0.009205	0.009251	0.009201	0.008677	0.010717	0.010781	0.010778	0.010782	0.011362
(176)	0.001	0.000818	0.000841	0.000827	0.000840	0.000815	0.001089	0.001142	0.001104	0.001142	0.001277
	0.0001	9.70E−05	9.20E−05	9.60E−05	9.20E−05	6.90E−05	0.000133	0.000138	0.000132	0.000140	0.000143
	1.00E−05	1.00E−05	9.00E−06	1.00E−05	9.00E−06	6.00E−06	2.00E−05	1.50E−05	2.00E−05	1.50E−05	1.30E−05
12 kb	0.05	0.047957	0.047567	0.047965	0.047565	0.045992	0.051783	0.051587	0.051810	0.051586	0.054699
	0.01	0.009034	0.009013	0.009044	0.009013	0.008269	0.010541	0.010506	0.010554	0.010505	0.011560
(235)	0.001	0.000849	0.000798	0.000849	0.000798	0.000700	0.001134	0.001104	0.001138	0.001104	0.001310
	0.0001	7.00E−05	6.70E−05	7.00E−05	6.70E−05	5.90E−05	0.000110	0.000108	0.000110	0.000108	0.000144
	1.00E−05	7.00E−06	4.00E−06	7.00E−06	4.00E−06	9.00E−06	1.20E−05	7.00E−06	1.20E−05	7.00E−06	1.90E−05
15 kb	0.05	0.048177	0.048027	0.048184	0.048027	0.045494	0.051983	0.052043	0.051979	0.052043	0.055872
	0.01	0.008980	0.009161	0.008981	0.009161	0.008136	0.010507	0.010668	0.010510	0.010668	0.012074
(293)	0.001	0.000789	0.000823	0.000791	0.000823	0.000685	0.001095	0.001105	0.001094	0.001105	0.001393
	0.0001	7.60E−05	8.00E−05	7.70E−05	8.00E−05	6.00E−05	0.000117	0.000133	0.000118	0.000133	0.000170
	1.00E−05	8.00E−06	5.00E−06	8.00E−06	5.00E−06	7.00E−06	1.20E−05	1.00E−05	1.20E−05	1.00E−05	2.20E−05
18 kb	0.05	0.048041	0.048115	0.048042	0.048115	0.044511	0.051962	0.052075	0.051965	0.052075	0.056474
	0.01	0.009191	0.009151	0.009190	0.009151	0.007883	0.010711	0.010740	0.010708	0.010740	0.012415
(352)	0.001	0.000815	0.000798	0.000816	0.000798	0.000637	0.001094	0.001083	0.001095	0.001083	0.001501
	0.0001	7.00E−05	7.50E−05	7.00E−05	7.50E−05	4.70E−05	0.000115	0.000112	0.000115	0.000112	0.000156

(Continues)

TABLE 2 (Continued)

Region size (# variants)	Nominal level α	Type I error rates of F-distributed statistics					Type I error rates of LRT statistics				
		FLMM (5)		FLMM (6)		LMM	FLMM (5)		FLMM (6)		LMM
		B-spline	Fourier	B-spline	Fourier	(1)	B-spline	Fourier	B-spline	Fourier	(1)
	1.00E−05	1.00E−05	7.00E−06	1.00E−05	7.00E−06	2.00E−06	1.10E−05	1.30E−05	1.10E−05	1.30E−05	2.20E−05
21 kb	0.05	0.047839	0.047807	0.047835	0.047807	0.043991	0.051618	0.051826	0.051613	0.051826	0.057561
	0.01	0.009033	0.008906	0.009033	0.008906	0.007682	0.010520	0.010481	0.010521	0.010481	0.012735
(410)	0.001	0.000800	0.000831	0.000800	0.000831	0.000639	0.001058	0.001112	0.001058	0.001112	0.001528
	0.0001	7.20E−05	6.80E−05	7.20E−05	6.80E−05	4.50E−05	0.000117	0.000122	0.000117	0.000122	0.000205
	1.00E−05	8.00E−06	1.00E−06	8.00E−06	1.00E−06	7.00E−06	1.10E−05	9.00E−06	1.10E−05	9.00E−06	2.20E−05
24 kb	0.05	0.048516	0.047988	0.048517	0.047988	0.043404	0.052276	0.051995	0.052278	0.051995	0.058415
	0.01	0.009040	0.009008	0.009040	0.009008	0.007647	0.010527	0.010607	0.010528	0.010607	0.013481
(469)	0.001	0.000817	0.000763	0.000817	0.000763	0.000633	0.001086	0.001061	0.001086	0.001061	0.001752
	0.0001	7.50E−05	6.30E−05	7.50E−05	6.30E−05	5.80E−05	0.000120	0.000107	0.000120	0.000107	0.000235
	1.00E−05	5.00E−06	3.00E−06	5.00E−06	3.00E−06	5.00E−06	1.30E−05	9.00E−06	1.30E−05	9.00E−06	3.20E−05
27 kb	0.05	0.047956	0.047670	0.047954	0.047670	0.042504	0.051746	0.051641	0.051744	0.051641	0.059221
	0.01	0.009087	0.008973	0.009087	0.008973	0.007321	0.010540	0.010534	0.010540	0.010534	0.013619
(527)	0.001	0.000807	0.000779	0.000807	0.000779	0.000577	0.001071	0.001062	0.001071	0.001062	0.001728
	0.0001	7.50E−05	7.00E−05	7.50E−05	7.00E−05	4.90E−05	0.000108	0.000128	0.000108	0.000128	0.000231
	1.00E−05	5.00E−06	4.00E−06	5.00E−06	4.00E−06	5.00E−06	1.00E−05	1.40E−05	1.00E−05	1.40E−05	3.30E−05
30 kb	0.05	0.047855	0.047912	0.047856	0.047912	0.041902	0.051689	0.051909	0.051690	0.051909	0.060004
	0.01	0.009045	0.008847	0.009045	0.008847	0.007145	0.010589	0.010473	0.010589	0.010473	0.014220
(586)	0.001	0.000801	0.000775	0.000801	0.000775	0.000565	0.001088	0.001043	0.001088	0.001043	0.001870
	0.0001	7.10E−05	7.30E−05	7.10E−05	7.30E−05	3.70E−05	0.000109	9.90E−05	0.000109	9.90E−05	0.000239
	1.00E−05	3.00E−06	5.00E−06	3.00E−06	5.00E−06	4.00E−06	1.10E−05	9.00E−06	1.10E−05	9.00E−06	2.90E−05

Note. FLMM: functional linear mixed model; LMM: linear mixed model; LRT: likelihood ratio test.

The order of B-spline basis was 4, the number of basis functions of B-spline was $K = K_\beta = 20$, and the number of Fourier basis functions was $K = K_\beta = 21$.

TABLE 3 Empirical type I error rates of the F-distributed statistics and LRT statistics at nominal levels $\alpha = 0.05, 0.01, 0.001, 0.0001$, and 0.00001 using the 75 two- or three-generation families with a total of 684 individuals as a template, when region sizes are 3,6 ..., 27,30 kb and all variants are rare

Region size (# variants)	Nominal level	Type I error rates of F-distributed statistics					Type I error rates of LRT statistics				
		FLMM (5)		FLMM (6)		LMM	FLMM (5)		FLMM (6)		LMM
	α	B-spline	Fourier	B-spline	Fourier	(1)	B-spline	Fourier	B-spline	Fourier	(1)
3 kb	0.05	0.011697	0.048875	0.040898	0.048524	0.049074	0.012473	0.051047	0.043001	0.050660	0.051550
	0.01	0.001889	0.009581	0.007882	0.009496	0.009570	0.002121	0.010464	0.008663	0.010380	0.010542
(53)	0.001	0.000126	0.000924	0.000713	0.000921	0.000921	0.000164	0.001118	0.000868	0.001117	0.001103
	0.0001	1.00E−05	7.60E−05	6.90E−05	7.40E−05	8.50E−05	1.50E−05	0.000111	8.60E−05	0.000106	0.000108
	1.00E−05	1.00E−06	1.00E−05	8.00E−06	1.00E−05	1.10E−05	2.00E−06	1.70E−05	1.30E−05	1.70E−05	1.60E−05
6 kb	0.05	0.034081	0.048315	0.046020	0.047672	0.048061	0.036685	0.051879	0.049387	0.051260	0.052117
	0.01	0.006175	0.009086	0.008688	0.008933	0.009108	0.007201	0.010569	0.010014	0.010375	0.010694
(106)	0.001	0.000540	0.000823	0.000768	0.000814	0.000815	0.000731	0.001090	0.001027	0.001072	0.001121
	0.0001	4.20E−05	7.60E−05	7.00E−05	8.70E−05	7.40E−05	6.60E−05	0.000122	0.000109	0.000128	0.000124
	1.00E−05	4.00E−06	8.00E−06	6.00E−06	8.00E−06	6.00E−06	8.00E−06	1.50E−05	9.00E−06	1.50E−05	1.30E−05
9 kb	0.05	0.046204	0.048242	0.048321	0.048256	0.047596	0.049718	0.052147	0.052003	0.052192	0.053262
	0.01	0.008830	0.009118	0.009304	0.009124	0.008925	0.010245	0.010711	0.010811	0.010719	0.011090
(159)	0.001	0.000807	0.000861	0.000844	0.000863	0.000798	0.001112	0.001151	0.001170	0.001150	0.001208
	0.0001	8.50E−05	9.00E−05	9.50E−05	9.30E−05	7.50E−05	0.000124	0.000136	0.000135	0.000136	0.000143
	1.00E−05	1.10E−05	9.00E−06	1.00E−05	9.00E−06	5.00E−06	2.00E−05	1.40E−05	2.30E−05	1.40E−05	1.20E−05
12 kb	0.05	0.047955	0.048358	0.048393	0.048342	0.046926	0.051767	0.052314	0.052231	0.052306	0.054044
	0.01	0.009163	0.009249	0.009250	0.009250	0.008645	0.010635	0.010815	0.010732	0.010810	0.011389
(212)	0.001	0.000835	0.000837	0.000847	0.000835	0.000741	0.001137	0.001139	0.001153	0.001140	0.001235
	0.0001	8.20E−05	7.60E−05	8.40E−05	7.60E−05	7.10E−05	0.000122	0.000123	0.000128	0.000123	0.000146
	1.00E−05	7.00E−06	7.00E−06	6.00E−06	7.00E−06	6.00E−06	1.80E−05	1.20E−05	1.80E−05	1.20E−05	1.50E−05
15 kb	0.05	0.047998	0.047907	0.048069	0.047897	0.046429	0.051968	0.051793	0.052052	0.051785	0.054614
	0.01	0.009110	0.009100	0.009130	0.009097	0.008611	0.010577	0.010758	0.010598	0.010758	0.011790
(265)	0.001	0.000844	0.000871	0.000846	0.000872	0.000718	0.001138	0.001153	0.001143	0.001154	0.001350
	0.0001	8.90E−05	9.00E−05	8.90E−05	9.10E−05	4.90E−05	0.000139	0.000131	0.000139	0.000132	0.000132
	1.00E−05	8.00E−06	6.00E−06	8.00E−06	6.00E−06	8.00E−06	1.30E−05	1.00E−05	1.30E−05	1.00E−05	2.00E−05
18 kb	0.05	0.048187	0.048045	0.048201	0.048046	0.045493	0.051922	0.052001	0.051946	0.052002	0.055095
	0.01	0.009141	0.009099	0.009147	0.009100	0.008331	0.010643	0.010660	0.010650	0.010661	0.011957

(Continues)

TABLE 3 (Continued)

Region size (# variants)	Nominal level	Type I error rates of F-distributed statistics					Type I error rates of LRT statistics				
		FLMM (5)		FLMM (6)		LMM	FLMM (5)		FLMM (6)		LMM
	α	B-spline	Fourier	B-spline	Fourier	(1)	B-spline	Fourier	B-spline	Fourier	(1)
(318)	0.001	0.000823	0.000801	0.000825	0.000801	0.000679	0.001109	0.001074	0.001110	0.001074	0.001356
	0.0001	7.20E−05	6.20E−05	7.30E−05	6.20E−05	5.60E−05	0.000125	0.000107	0.000127	0.000107	0.000164
	1.00E−05	5.00E−06	3.00E−06	5.00E−06	3.00E−06	7.00E−06	1.50E−05	7.00E−06	1.50E−05	7.00E−06	2.10E−05
21 kb	0.05	0.047950	0.048063	0.047966	0.048063	0.045322	0.051684	0.052039	0.051692	0.052039	0.056408
	0.01	0.008960	0.009025	0.008955	0.009025	0.008135	0.010468	0.010670	0.010469	0.010670	0.012498
(371)	0.001	0.000835	0.000772	0.000835	0.000772	0.000670	0.001095	0.001084	0.001095	0.001084	0.001456
	0.0001	7.50E−05	7.80E−05	7.50E−05	7.80E−05	5.00E−05	0.000119	0.000119	0.000119	0.000119	0.000168
	1.00E−05	8.00E−06	7.00E−06	8.00E−06	7.00E−06	5.00E−06	1.10E−05	1.00E−05	1.10E−05	1.00E−05	1.50E−05
24 kb	0.05	0.048216	0.048099	0.048209	0.048099	0.045004	0.052112	0.052018	0.052105	0.052018	0.057104
	0.01	0.008985	0.008994	0.008984	0.008994	0.008057	0.010551	0.010546	0.010551	0.010546	0.012826
(424)	0.001	0.000820	0.000860	0.000820	0.000860	0.000727	0.001070	0.001174	0.001071	0.001174	0.001591
	0.0001	7.20E−05	7.00E−05	7.20E−05	7.00E−05	5.10E−05	0.000106	0.000106	0.000106	0.000106	0.000216
	1.00E−05	5.00E−06	1.10E−05	5.00E−06	1.10E−05	7.00E−06	1.30E−05	1.50E−05	1.30E−05	1.50E−05	2.60E−05
27 kb	0.05	0.047998	0.047927	0.047995	0.047927	0.043921	0.051744	0.051893	0.051743	0.051893	0.057692
	0.01	0.009037	0.009023	0.009037	0.009023	0.007595	0.010633	0.010649	0.010633	0.010649	0.012803
(477)	0.001	0.000859	0.000793	0.000859	0.000793	0.000640	0.001129	0.001094	0.001129	0.001094	0.001583
	0.0001	7.40E−05	7.40E−05	7.40E−05	7.40E−05	4.80E−05	0.000114	0.000111	0.000114	0.000111	0.000185
	1.00E−05	2.00E−06	8.00E−06	2.00E−06	8.00E−06	5.00E−06	1.00E−05	1.30E−05	1.00E−05	1.30E−05	2.40E−05
30 kb	0.05	0.047870	0.047953	0.047869	0.047953	0.043784	0.051694	0.051866	0.051694	0.051866	0.058713
	0.01	0.009105	0.009155	0.009105	0.009155	0.007572	0.010618	0.010696	0.010618	0.010696	0.013239
(530)	0.001	0.000804	0.000793	0.000803	0.000793	0.000642	0.001092	0.001065	0.001093	0.001065	0.001641
	0.0001	5.70E−05	6.90E−05	5.70E−05	6.90E−05	5.90E−05	0.000104	0.000116	0.000104	0.000116	0.000227
	1.00E−05	5.00E−06	4.00E−06	5.00E−06	4.00E−06	2.00E−06	9.00E−06	9.00E−06	9.00E−06	9.00E−06	3.20E−05

Note. FLMM: functional linear mixed model; LMM: linear mixed model; LRT: likelihood ratio test.

The order of B-spline basis was 4, the number of basis functions of B-spline was $K = K_\beta = 20$, and the number of Fourier basis functions was $K = K_\beta = 21$.

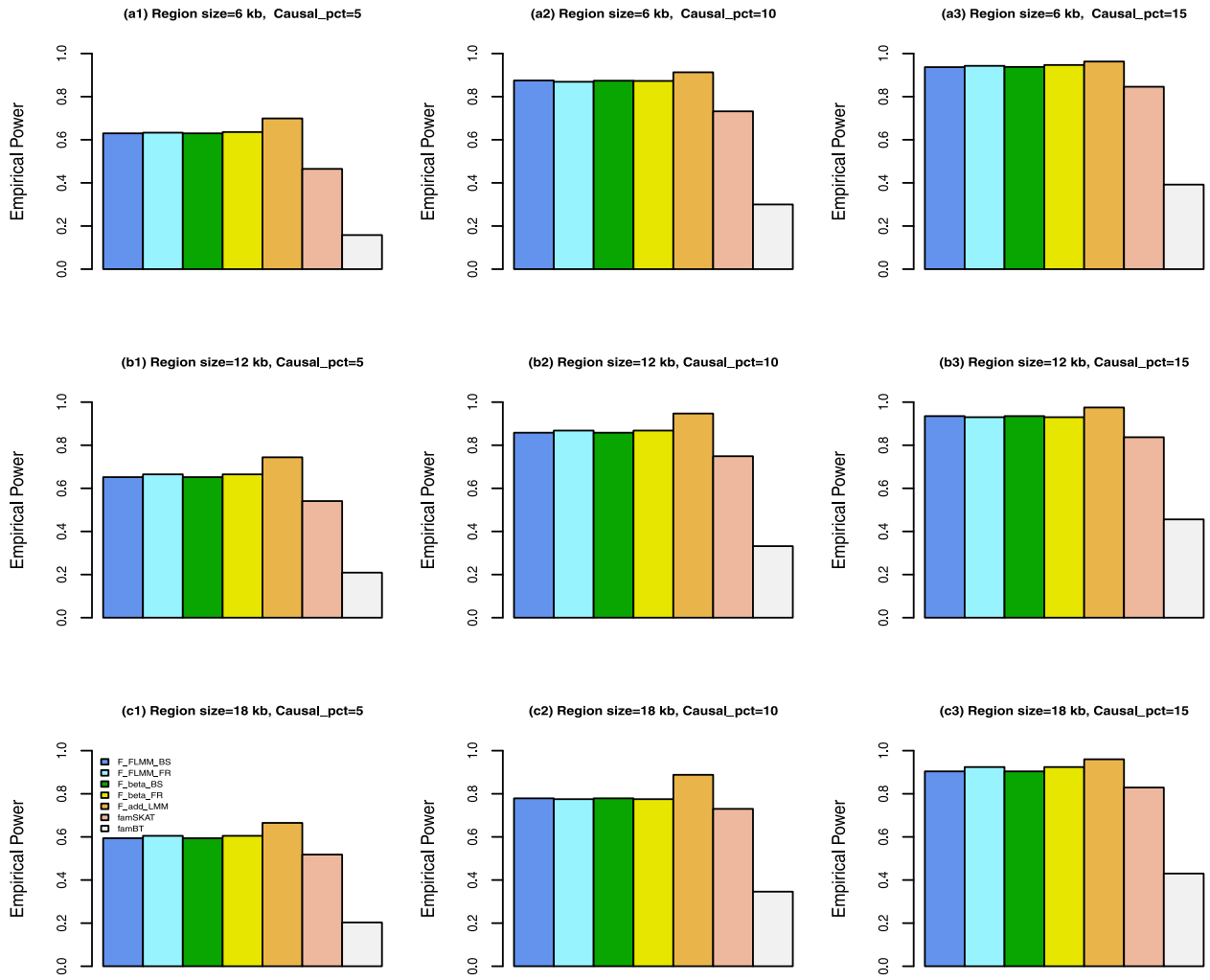

FIGURE 1 The empirical power of the F-test statistics, famSKAT, and famBT at $\alpha = 0.001$ using the 75 two- or three-generation families with a total of 684 individuals as a template, when some variants are common and the rest are rare, 20% causal variants have negative effects, and the region sizes are 6, 12, and 18 kb, respectively. *Note*. The order of B-spline basis was 4, the number of basis functions of B-spline was $K = K_\beta = 20$, and the number of Fourier basis functions was $K = K_\beta = 21$. Causal_pct: percentage of causal variants; famBT: family-based burden test; famSKAT: family-based sequence kernel association test

nominal significance levels $\alpha = 0.05$, 0.01, 0.001, 0.0001, and 0.00001.

In Tables 2 and 3, the results are based on the template C of 75 families. The empirical type I error rates of the F-test statistics of the LMM (1) and FLMMs (5) and (6) are generally lower than the nominal levels. Hence, the F-test statistics are conservative and control the type I error rates correctly, no matter whether the genotype data are smoothed or not or which basis functions are used to smooth the GVF and $\beta(t)$ or if both rare and common variants are used or only rare variants are used. The empirical type I error rates of the LRT statistics of the FLMMs (5) and (6) are around the nominal levels at $\alpha = 0.05$, 0.01, 0.001, and 0.0001 levels, but can be higher than the $\alpha = 0.00001$ nominal level. As the region size and number of variants increase, the type I error rates at the nominal level $\alpha = 0.00001$ of the LRT statistics of the FLMMs (5) and (6) are gradually become closer to 0.00001. The empirical type I error rates of the LRT statistics of the LMM (1) are generally higher than the nominal levels.

In Tables A.1 and A.2 of Supporting Information, we show the type I error rates using the template B of 50 families. The empirical type I error rates of the F-test statistics of the LMM (1) and FLMMs (5) and (6) are lower than the nominal levels, and the F-test statistics are conservative. The empirical type I error rates of the LRT statistics of the FLMMs (5) and (6) are around the nominal levels at 0.05, 0.01, and 0.001 levels, but can be higher than

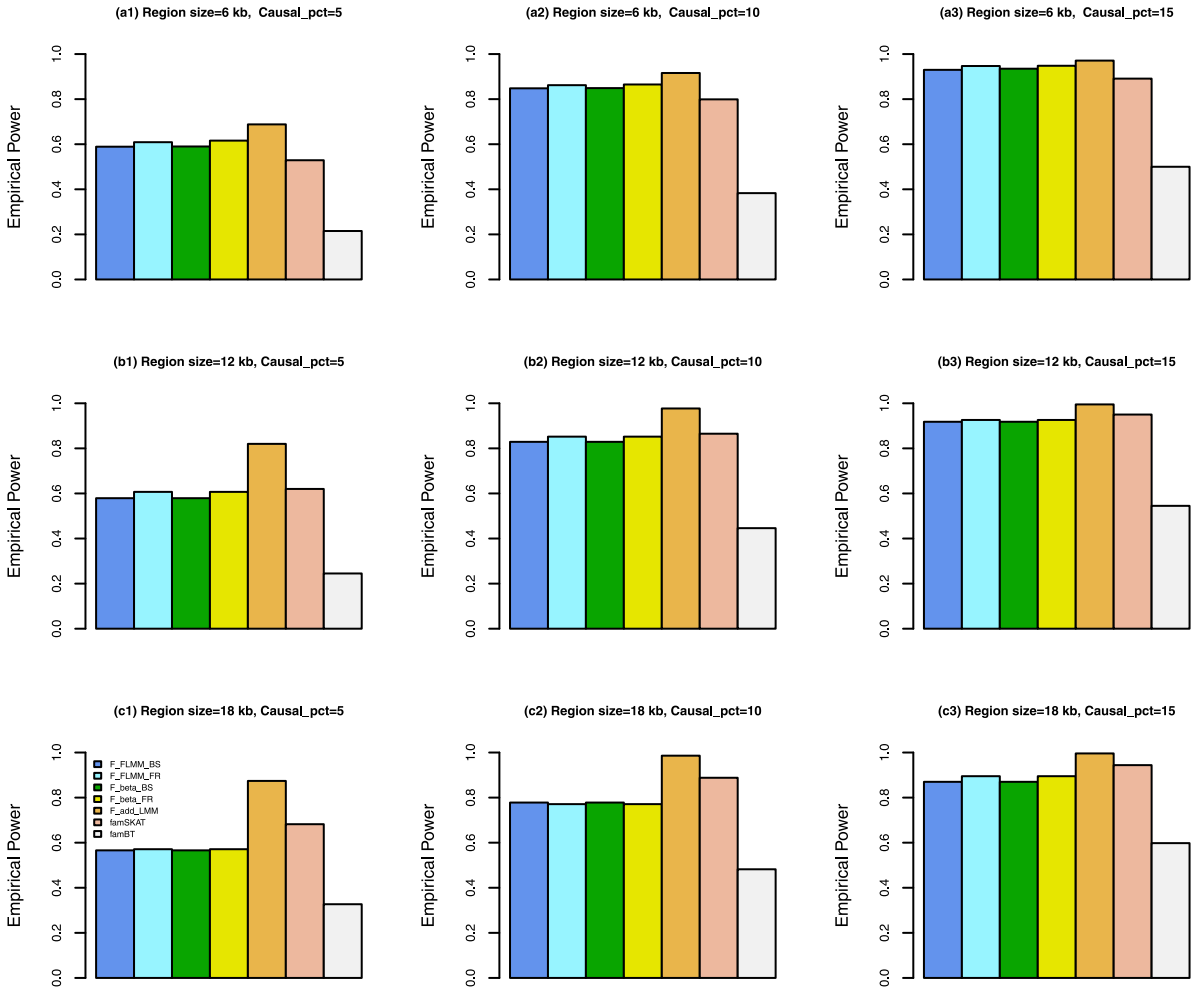

FIGURE 2 The empirical power of the F-test statistics, famSKAT, and famBT at $\alpha = 0.001$ using the 75 two- or three-generation families with a total of 684 individuals as a template, when all variants are rare, 20% causal variants have negative effects, and the region sizes are 6, 12, and 18 kb, respectively. *Note*. The order of B-spline basis was 4, the number of basis functions of B-spline was $K = K_\beta = 20$, the number of Fourier basis functions was $K = K_\beta = 21$. Causal_pct means percentage of causal variants; famBT: family-based burden test; famSKAT: family-based sequence kernel association test

the nominal levels when $\alpha = 0.0001$ and 0.00001. The empirical type I error rates of the LRT statistics of the LMM (1) are generally higher than the nominal levels.

In Tables A.3 and A.4 of Supporting Information, we show the type I error rates using the template A of 25 families. The empirical type I error rates of the F-test statistics of the LMM (1) and FLMMs (5) and (6) are lower than the nominal levels, and the F-test statistics are conservative. The empirical type I error rates of the LRT statistics of the FLMMs (5) and (6) are around the nominal levels at 0.05 and 0.01 levels, but can be higher than the nominal levels when $\alpha = 0.001$, 0.0001, and 0.00001.

3.1.2 | Empirical power simulations using the template C of 75 families

Based on the simulated sequence data, the power of the F-test statistics was compared with the power of the famSKAT and famBT statistics. Figures 1 and 2 report the results when 20%/80% causal variants have negative/positive effects and region sizes are 6, 12, and 18 kb. In Figure 1, some variants are common and the rest are rare, and the variants are all rare in Figure 2. In Supporting Information, we report more results in Figures A.1–A.10 when some variants are common and the rest are rare and in Figures A.11 – A.20 when the variants are all rare. In Plots (a1)–(a3) of Figures A.1 – A.20, all causal variants have positive effects; when 20%/80% causal variants have negative/positive effects, we present the results in Plots (b1) – (b3); when 50%/50% causal variants have negative/positive effects, the results are presented in Plots (c1) – (c3). Therefore, the results of Figure 1 are Plots (b1) – (b3) in Figures A.2, A.4, and A.6, respectively, and the results of Figure 2 are Plots (b1) – (b3) in Figures A.12, A.14, and A.16, respectively.

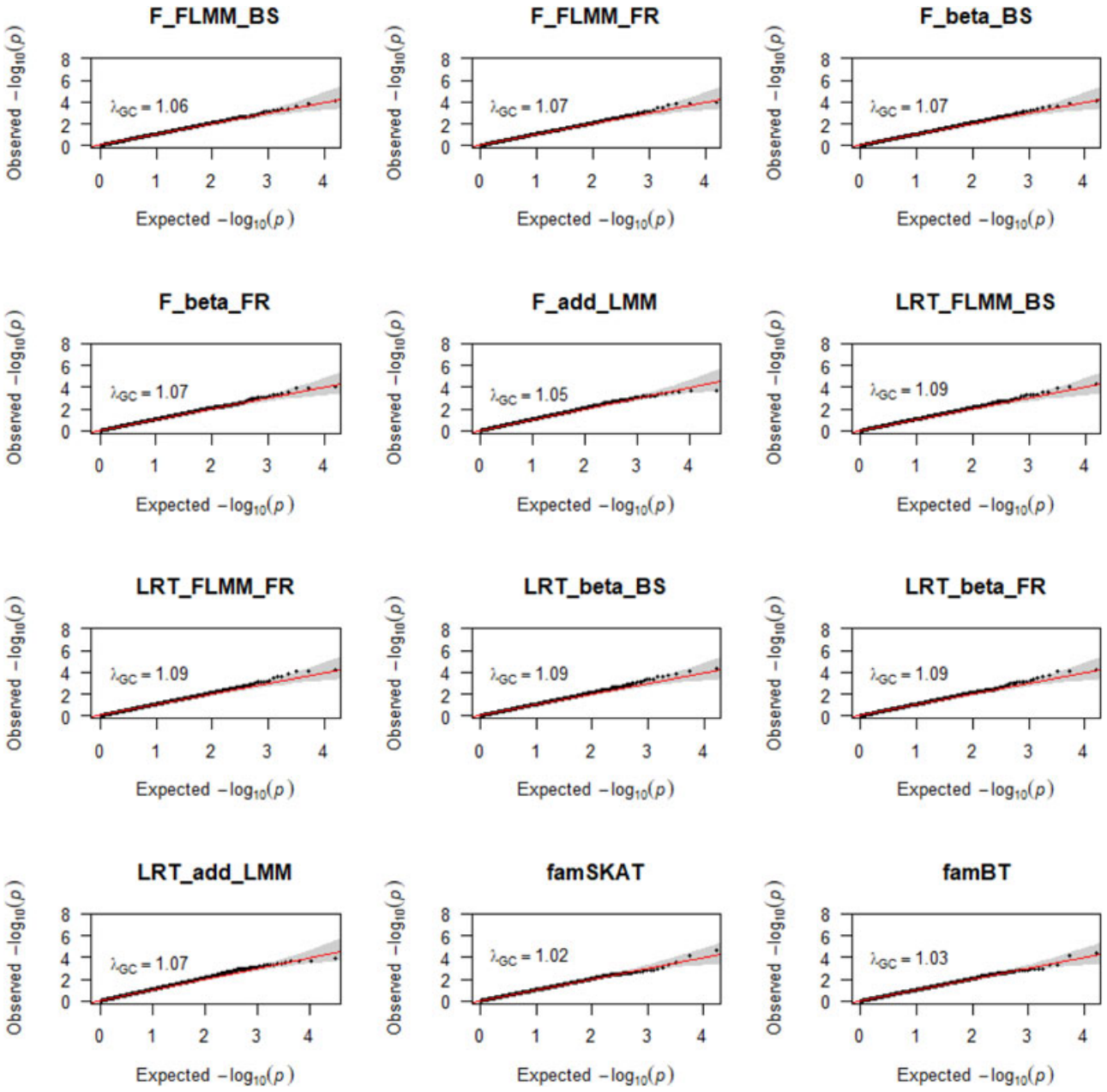

FIGURE 3 *Q*–*Q* plots for the *F*-test and LRT statistics, famSKAT, and famBT for the Myopia Family Study data. F_add_LMM: F-test of additive LMM (1) vs. null model (9); F_beta_BS: F-test of FLMM (6) with the B-Spline basis vs. null model (9); F_beta_FR: F-test of FLMM (6) with the Fourier basis vs. null model (9); F_FLMM_BS: F-test of FLMM (5) with the B-Spline basis vs. null model (9); F_FLMM_FR: F-test of FLMM (5) with the Fourier basis vs. null model (9); famBT: family-based burden tests; famSKAT: family-based sequence kernel association test; FLMMs: functional linear mixed models; GVF: genetic variant function; LMMs: linear mixed models; LRT: likelihood ratio test

When some variants are common and the rest are rare, the *F*-test statistics of LMM (1) and FLMMs (5) and (6) have higher power than the kernel and burden tests, famSKAT and famBT, respectively, in Figures 1 and A.1–A.10. The four *F*-test statistics of FLMMs (5) and (6) perform similarly, whereas the *F*-test statistic of LMM (1) performs the best. If all variants are rare and the region sizes are 3 and 6 kb, the *F*-test statistics of LMM (1) and FLMMs (5) and (6) have similar power as famSKAT in Plots (a1) – (a3) of Figure 2, and Figures A.11 and A.12. If all variants are rare and the region sizes are between 9 and 30 kb, the *F*-test statistic of LMM (1) has the highest power, whereas famSKAT performs similarly to or better than the *F*-test statistics of FLMMs (5) and (6) in Plots (b1) – (b3) and (c1) – (c3) of Figure 2, and Figures A.13 – A.20.

The high power levels of the *F*-test statistic of LMM (1) in Figures 2 and A.11–A.20 show that the LMM (1) is useful in analyzing rare variants. When some variants are

TABLE 4 Results of association analysis of refractive error data in the Myopia Family Study

Chr	Gene	Start	End	Number of variants	F-distributed statistics					LRT statistics					
					FLMM (5)		FLMM (6)		Additive	FLMM (5)		FLMM (6)		Additive	
					B-spline	Fourier	B-spline	Fourier	LMM (1)	B-spline	Fourier	B-spline	Fourier	LMM (1)	famSKAT	famBT
11	*NAV2*	19,569,563	20,129,311	13	8.24E−05	9.07E−05	8.24E−05	9.07E−05	0.002558	4.86E−05	5.15E−05	4.86E−05	5.15E−05	0.001704	0.317707	0.554589
1	*HSPG2;CELA3B*	22,265,293	22,296,307	12	0.000144	0.000332	0.000144	0.000332	0.000625	8.97E−05	0.000215	8.97E−05	0.000215	0.000414	2.26E−05	3.66E−05
1	*HP1BP3*	21,070,238	21,102,799	10	0.000212	0.000137	0.000212	0.000137	0.000212	0.000137	8.92E−05	0.000137	8.92E−05	0.000137	0.193372	0.190859
4	*TEC*	48,138,235	48,270,152	44	0.000388	0.000141	0.000367	0.000126	0.001796	0.000264	8.35E−05	0.000249	7.40E−05	0.000864	0.018546	0.752173
1	*SLC9A1;WDTC1*	27,493,679	27,558,522	13	0.000404	0.000519	0.000404	0.000519	0.001093	0.000276	0.000350	0.000276	0.000350	0.000763	0.445853	0.948234
11	*CADM1; LOC101928985*	115,515,326	115,552,918	2	0.000557	0.003393	0.000557	0.000557	0.000557	0.000459	0.003141	0.000459	0.000459	0.000459	0.355841	0.355585

Note. Chr: chromosome; famBT: family-based burden test; famSKAT: family-based sequence kernel association test; FLMM: functional linear mixed model; LMM: linear mixed model; LRT: likelihood ratio test. The association results were included if a p-value is smaller or around 10^{-3}.

common and the rest are rare, the LMM (1) is also powerful especially in the presence of a large number of variants. The famSKAT has higher power than the burden test famBT. The four F-test statistics of FLMMs (5) and (6) have similar good power levels. The power levels of the F-test statistics of beta-smooth only FLMM (6) are almost identical to those of the F-test statistics of FLMM (5), which smooth both the GVFs $X_i(u)$ and the genetic effect function $\beta(t)$, regardless of basis choice. Hence, the four F-test statistics of FLMMs (5) and (6) are very stable in terms of power performance and they do not strongly depend on whether the genotype data are smoothed or not, nor on which basis function is used.

3.2 | Analysis of refractive error data in the Myopia Family Study

We carried out gene-based tests to investigate genes on autosomes that may affect the variation of refractive error using the F-test and LRT statistics, the family kernel-based (famSKAT), and burden test (famBT; Chen et al., 2013). Quantile–quantile (Q–Q) plots of the gene-based statistics in Figure 3 show that the F-test and LRT statistics, famSKAT, and famBT statistics had similar λ_{GC} values. In Table 4, the strongest association was detected between refractive error and *NAV2* with p-values of FLMMs (5) and (6) tests <0.0001, and associations were detected for genes *HSPG2; CELA3B*, *HP1BP3*, *TEC*, *SLC9A1;WDTC1*, and *CADM1; LOC101928985* with p-values of LMM (1) and FLMMs (5) and (6) tests are smaller than or around 0.001. Interestingly, famSKAT and famBT show an association signal at the gene *HSPG2;CELA3B*, but not at the others. We note that none of the genes shows a significant association after a Bonferroni correction $0.05/8282 = 6.04 \times 10^{-6}$ in this moderately sized sample that includes 36 pedigrees and 300 genotyped/phenotyped individuals.

Quantile–quantile (Q–Q) plots of the gene-based statistics in Figure 3 show that the F-test and LRT statistics, famSKAT, and famBT statistics had similar λ_{GC} values.

4 | DISCUSSION

In this paper we develop tests based on LMMs and FLMMs for gene-based tests of association between a quantitative trait and genetic variants on pedigrees. In the models, the effect of a major gene is modeled as a fixed effect, the contribution of polygenes is modeled as a separate random variation, and the correlation of pedigree members is modeled by inbreeding/kinship coefficients. Cholesky decomposition is utilized to make the traits standard normal. Then, F-distributed statistics and LRT statistics based on the LMMs and FLMMs are

built to test for association between the quantitative trait and the genetic variants. By simulation, we show that the F distributed statistics are conservative and control type I errors correctly. The proposed models are useful in whole genome and whole exome association studies of complex traits.

The F-test statistics of LMMs have similar or higher power than the FLMMs, kernel-based famSKAT, and burden test famBT. The FLMMs perform well when analyzing a combination of rare and common variants. The kernel-based famSKAT performs better than burden test famBT. In our previous work, we showed that the tests of fixed effect regression models have higher power than SKAT for population data in major gene association studies (Fan, Chiu, et al., 2016). Therefore, our models provide an alternative competitive method for carrying out gene-based association tests based on next-generation sequencing data.

For small samples of only 25 pedigrees (template A), the LRT statistics of the FLMMs (5) and (6) control type I errors correctly at 0.05 and 0.01 levels, but can inflate type I errors when $\alpha = 0.001$, 0.0001, and 0,00001 when the number of B-spline basis functions was $K = K_\beta = 20$, and the number of Fourier basis functions was $K = K_\beta = 21$. Hence, the LRT statistics of the FLMMs (5) and (6) can be used in candidate gene analysis for small samples. When the sample sizes increase, the LRT statistics of the FLMMs (5) and (6) control the type I error rates at lower levels = 0.001; 0.0001; and they can be used in genome-wide or exome-wide analysis. The empirical type I error rates of the LRT statistics of the LMM (1) are generally higher than the nominal levels.

In Svishcheva et al. (2015), FLMMs were proposed to test association using F-distributed statistics which are essentially our Models (2) and (3). However, our LMM (1) was not included in Svishcheva et al. (2015), which actually performs the best among the models we considered. In addition, we examine the performance of the LRT statistics of LMMs and FLMMs, and show the LRT statistics of FLMMs are useful in candidate gene analysis for small samples and are useful in genome-wide or exome-wide analysis if the sample sizes are moderate or large.

COMPUTER PROGRAM

The methods proposed in this paper are implemented using fda procedures implemented in the statistical package R. The R codes for data analysis and simulations are available from the web https://sites.google.com/a/georgetown.edu/ruzong-fan/about.

ACKNOWLEDGMENTS

This study was supported by the Intramural Research Program of the National Human Genome Research Institute (A. F. W. and J. E. B.-W.) and by the Intramural Research Program of the National Institute of Mental Health (F. J. M. M.), National Institutes of Health (NIH), Bethesda, MD. This study was also supported by Wei Chen's NIH grant (R01EY024226) and Yunnan Applied Basic Research Projects China, Yunnan Province, P. R. China (U0120170557). This study utilized the high-performance computational capabilities of the Biowulf/Linux cluster at the NIH, Bethesda, MD (http://biowulf.nih.gov).

ORCID

Daniel E. Weeks https://orcid.org/0000-0001-9410-7228
Alexander F. Wilson https://orcid.org/0000-0002-6682-8156
Joan E. Bailey-Wilson https://orcid.org/0000-0002-9153-2920
Ruzong Fan http://orcid.org/0000-0002-7603-2135

REFERENCES

Abecasis, G. R., Auton, A., Brooks, L. D., DePristo, M. A., Durbin, R. M., & Handsaker, R. E., 1000 Genomes Project Consortium (2012). An integrated map of genetic variation from 1,092 human genomes. *Nature*, *491*, 56–65.

Amos, C. I. (1994). Robust variance-components approach for assessing linkage in pedigrees. *American Journal of Human Genetics*, *54*, 534–543.

Astle, W., & Balding, D. J. (2009). Population structure and cryptic relatedness in genetic association studies. *Statistical Science*, *24*, 451–471.

Aulchenko, Y. S., de Koning, D. J., & Haley, C. (2007). Genomewide rapid association using mixed model and regression: A fast and simple method for genomewide pedigreebased quantitative trait loci association analysis. *Genetics*, *177*, 577–585.

Chen, H., Meigs, J. B., & Dupuis, J. (2013). Sequence kernel association test for quantitative traits in family samples. *Genetic Epidemiology*, *37*(2), 196–204.

de Boor, C. (2001). *A practical guide to splines*. Applied Mathematical Sciences (Vol. 27, revised version). New York: Springer.

Fan, R. Z., Wang, Y. F., Mills, J. L., Wilson, A. F., Bailey-Wilson, J. E., & Xiong, M. M. (2013). Functional linear models for association analysis of quantitative traits. *Genetics Epidemiology*, *37*, 726–742.

Fan, R. Z., Wang, Y. F., Mills, J. L., Carter, T. C., Lobach, I., Wilson, A. F., & Xiong, M. M. (2014). Generalized functional linear models for case-control association studies. *Genetics Epidemiology*, *38*, 622–637.

Fan, R. Z., Wang, Y. F., Qi, Y., Ding, Y., Weeks, D. E., Lu, Z. H., & Chen, W. (2016). Gene-based association analysis for censored

traits via functional regressions. *Genetics Epidemiology*, *40*, 133–143.

Fan, R. Z., Chiu, C. Y., Jung, J. S., Weeks, D. E., Wilson, A. F., Bailey-Wilson, J. E., & Xiong, M. M. (2016). A comparison study of fixed and mixed effect models for gene level association studies of complex traits. *Genetics Epidemiology*, *40*, 702–721.

Ferraty, F., & Romain, Y. (2010). *The oxford handbook of functional data analysis*. New York: Oxford University Press.

Han, F., & Pan, W. (2010). A data-adaptive sum test for disease association with multiple common or rare variants. *Human Heredity*, *70*, 42–54.

Horváth, L., & Kokoszka, P. (2012). *Inference for functional data with applications*. New York: Springer.

Ionita-Laza, I., Lee, S., Makarov, V., Buxbaum, J. D., & Lin, X. (2013). Sequence kernel association tests for the combined effect of rare and common variants. *American Journal of Human Genetics*, *92*, 841–853.

Jiang, Y. D., Chiu, C. Y., Yan, Q., Chen, W., Gorin, M. B., Conley, Y. P., ... Fan, R. Z.(2018). Gene-based association testing of dichotomous traits with generalized functional linear mixed models using extended pedigrees. Manuscript.

Kang, H. M., Sul, J. H., Service, S. K., Zaitlen, N. A., Kong, S. Y., Freimer, N. B., & Eskin, E. (2010). Variance component model to account for sample structure in genome-wide association studies. *Nature Genetics*, *42*, 348–354.

Korte, A., Vilhjalmsson, B. J., Segura, V., Platt, A., Long, Q., & Nordborg, M. (2012). A mixed-model approach for genomewide association studies of correlated traits in structured populations. *Nature Genetics*, *44*, 1066–1071.

Lange, K. (2002). *Mathematical and statistical methods for genetic analysis* (2nd ed.). Berlin, Heidelberg, New York: Springer.

Lee, S., Emond, M. J., Bamshad, M. J., Barnes, K. C., Rieder, M. J., Nickerson, D. A., & Lin, X. (2012). Optimal unified approach for rare-variant association testing with application to small-sample case-control whole-exome sequencing studies. *American Journal of Human Genetics*, *91*, 224–237.

Lek, M., Karczewski, K. J., Minikel, E. V., Samocha, K. E., Banks, E., & Fennell, T., Exome Aggregation Consortium (2016). Analysis of protein-coding genetic variation in 60,706 humans. *Nature*, *536*, 285–291.

Li, B., & Leal, S. M. (2008). Methods for detecting associations with rare variants for common diseases: Application to analysis of sequence data. *American Journal of Human Genetics*, *83*, 311–321.

Lippert, C., Listgarten, J., Liu, Y., Kadie, C. M., Davidson, R. I., & Heckerman, D. (2011). FaST linear mixed models for genome-wide association studies. *Nature Methods*, *8*, 833–835.

Listgarten, J., Lippert, C., Kadie, C. M., Davidson, R. I., Eskin, E., & Heckerman, D. (2012). Improved linear mixed models for genome-wide association studies. *Nature Methods*, *9*, 525–526.

Luo, L., Boerwinkle, E., & Xiong, M. M. (2011). Association studies for next-generation sequencing. *Genome Research*, *21*, 1099–1108.

Luo, L., Zhu, Y., & Xiong, M. M. (2012). Quantitative trait locus analysis for next-generation sequencing with the functional linear models. *Journal of Medical Genetics*, *49*, 513–524.

Luo, L., Zhu, Y., & Xiong, M. M. (2013). Smoothed functional principal component analysis for testing association of the entire allelic spectrum of genetic variation. *European Journal of Human Genetics*, *21*, 217–224.

Madsen, B. E., & Browning, S. R. (2009). A groupwise association test for rare mutations using a weighted sum statistic. *PLOS Genetics*, *5*, e1000384.

Morgenthaler, S., & Thilly, W. G. (2007). A strategy to discover genes that carry multi-allelic or mono-allelic risk for common diseases: A cohort allelic sums test (CAST). *Mutation Research*, *615*, 28–56.

Morris, A. P., & Zeggini, E. (2010). An evaluation of statistical approaches to rare variant analysis in genetic association studies. *Genetic Epidemiology*, *34*, 188–193.

Musolf, A. M., Simpson, C. L., Moiz, B. A., Long, K. A., Portas, L., Murgia, F., & Bailey-Wilson, J. E. (2017). Caucasian families exhibit significant linkage of myopia to chromosome 11p. *Investigative Ophthalmology & Visual Science*, *59*(9), 3547–3554.

Musolf, A. M., Simpson, C. L., Long, K. A., Moiz, B. A., Lewis, D. D., Middlebrooks, C. D., & Stambolian, D. (2018). Myopia in Chinese families shows linkage to 10q26.13. *Molecular Vision*, *24*, 29–42.

Oualkacha, K., Dastani, Z., Li, R., Cingolani, P. E., Spector, T. D., Hammond, C. J., & Greenwood, C. M. (2013). Adjusted sequence kernel association test for rare variants controlling for cryptic and family relatedness. *Genetic Epidemiology*, *37*(4), 366–376.

Price, A. L., Kryukov, G. V., de Bakker, P. I. W., Purcell, S. M., Staples, J., Wei, L. J., & Sunyaev, S. R. (2010). Pooled association tests for rare variants in exon-resequencing studies. *American Journal of Human Genetics*, *86*, 832–838.

The R Project for Statistical Computing (https://www.r-project.org/)

Ramsay, J. O., Hooker, G., & Graves, S. (2009). *Functional data analysis with R and Matlab*. New York: Springer.

Ramsay, J. O., & Silverman, B. W. (2005). *Functional data analysis* (2nd ed.). New York: Springer.

Ross, S. M. (1996). *Stochastic processes* (2nd ed.). New York: John Wiley & Sons.

Rusk, N., & Kiermer, V. (2008). Primer: Sequencing the next generation. *Nature Methods*, *5*, 15.

Schaffner, S. F., Foo, C., Gabriel, S., Reich, D., Daly, M. J., & Altshuler, D. (2005). Calibrating a coalescent simulation of human genome sequence variation. *Genome Research*, *15*, 1576–1583.

Schifano, E. D., Epstein, M. P., Bielak, L. F., Jhun, M. A., Kardia, S. L., Peyser, P. A., & Lin, X. (2012). SNP set association analysis for familial data. *Genetic Epidemiology*, *36*(8), 797–810.

Svishcheva, G. R., Belonogova, N. M., & Axenovich, T. I. (2015). Region-based association test for familial data under functional linear models. *PLOS ONE*, *10*, e0128999.

Tennessen, J. A., Bigham, A. W., O'Connor, T. D., Fu, W., Kenny, E. E., Gravel, S. ... NHLBI Exome Sequencing Project (2012). Evolution and functional impact of rare coding variation from deep sequencing of human exomes. *Science*, *337*, 64–69.

The International HapMap Consortium (2007). A second generation human haplotype map of over 3.1 million SNPs. *Nature*, *449*, 851–861.

Vsevolozhskaya, O. A., Zaykin, D. V., Greenwood, M. C., Wei, C., & Lu, Q. (2014). Functional analysis of variance for association studies. *PLOS ONE*, *9*, e105074.

Vsevolozhskaya, O. A., Zaykin, D. V., Barondess, D. A., Tong, X., Jadhav, S., & Lu, Q. (2016). Uncovering local trends in genetic

effects of multiple phenotypes via functional linear models. *Genetics Epidemiology*, *40*, 210–221.

Wojciechowski, R. J., Bailey-Wilson, J. E., & Stambolian, D. (2009). Fine-mapping of candidate region in Amish and Ashkenazi families confirms linkage of refractive error to a QTL on 1p34-p36. *Molecular Vision*, *15*, 1398–406.

Wojciechowski, R. J., Stambolian, D., Ciner, E. B., Ibay, G., Holmes, T. N., & Bailey-Wilson, J. E. (2009). Genomewide linkage scans for ocular refraction and meta-analysis of four populations in the Myopia Family Study. *Investigative Ophthalmology & Visual Science*, *50*(5), 2024–2032.

Wu, M. C., Lee, S., Cai, T., Li, Y., Boehnke, M., & Lin, X. (2011). Rare-variant association testing for sequencing data with the sequence kernel association test. *American Journal of Human Genetics*, *89*, 82–93.

Yang, J., Zaitlen, N. A., Goddard, M. E., Visscher, P. M., & Price, A. L. (2014). Advantages and pitfalls in the application of mixed-model association methods. *Nature Genetics*, *46*, 100–106.

Yu, J., Pressoir, G., Briggs, W. H., Vroh Bi, I., Yamasaki, M., Doebley, J. F., & Holland, J. B. (2006). A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nature Genetics*, *38*, 203–208.

Zhou, X., & Stephens, M. (2012). Genome-wide efficient mixed-model analysis for association studies. *Nature Genetics*, *44*, 821–824.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Chiu C-y, Yuan F, Zhang B, et al. Linear mixed models for association analysis of quantitative traits with next-generation sequencing data. *Genet. Epidemiol.* 2019;43:189-206. https://doi.org/10.1002/gepi.22177