A Sequence Kernel Association Test for Dichotomous Traits in Family Samples under a Generalized Linear Mixed Model

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Key Words

Dichotomous traits · Family samples · Generalized linear mixed model · Linear kernel function · Sequence data

Abstract

Objective: The existing methods for identifying multiple rare variants underlying complex diseases in family samples are underpowered. Therefore, we aim to develop a new set-based method for an association study of dichotomous traits in family samples. **Methods:** We introduce a framework for testing the association of genetic variants with diseases in family samples based on a generalized linear mixed model. Our proposed method is based on a kernel machine regression and can be viewed as an extension of the sequence kernel association test (SKAT and famSKAT) for application to family data with dichotomous traits (F-SKAT). **Results:** Our simulation studies show that the original SKAT has inflated type I error rates when applied directly to family data. By contrast, our proposed F-SKAT has the correct type I error rate. Furthermore, in all of the considered scenarios, F-SKAT, which uses all family data, has higher power than both SKAT, which uses only unrelated individuals from the family data, and another method, which uses all family data. **Conclusion:** We propose a set-based association test that can be used to

analyze family data with dichotomous phenotypes while handling genetic variants with the same or opposite directions of effects as well as any types of family relationships.

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Introduction

With recent advances in high-throughput sequencing technology, significant progress has been made in identifying the association between genetic variants and complex diseases [1, 2]. Such progress requires appropriate study designs and statistical methods. Genome-wide association studies (GWASs) have been widely used both for identifying common single-nucleotide polymorphisms (SNPs) associated with human diseases and for further understanding the genetic basis of complex diseases [3–7]. In a typical GWAS, hundreds or thousands of individuals are recruited, and a large number of genetic markers are genotyped for all of the subjects. The association between the trait and each of the genetic markers is usually tested one by one through single-marker association tests. As a useful complement to the single-marker test, gene-based (or, more generally, set-based) tests are becoming more attractive [8–10]. If there are multiple causative variants

which have small individual effects, a single-marker analysis may not identify those weak signals. On the other hand, set-based tests have higher power because they combine the effects of all SNPs in the set and thus may be able to detect small effects. In addition, set-based tests may have higher power in the presence of genetic heterogeneity. And finally, set-based approaches greatly reduce the burden of multiple testing in GWASs.

Many set-based approaches have been developed in recent years [8, 9, 11–22]. One popular test is the sequence kernel association test (SKAT) [9, 21, 22], a flexible, computationally efficient, and regression-based approach. In SKAT, covariates can be easily incorporated into the model. In addition, predefined weights can be assigned to each SNP in the SNP set. This increases the power when prior information shows that certain types of markers may be associated with a trait. For example, a weight as a function of a minor allele frequency (MAF) that follows a beta density is proposed in Wu et al. [21], which assumes that rare genetic variants have larger effects on common diseases. Furthermore, the test statistic derived in SKAT follows a mixture of χ^2 distributions. Thus, the p values can be computed analytically without permutation, leading to a significant improvement in computation.

Family-based designs have been widely used to study the association between diseases and genetic variants [23–26]. In GWASs with unrelated samples, a general linear model is usually used to investigate the association between quantitative phenotypes and genetic markers. However, a general linear model results in an inflated type I error rate when the family correlation is not appropriately handled. Thus, instead of a general linear model, a linear mixed model including a random effect is usually employed to deal with correlation between family samples. The covariance of random effects of all individuals can be expressed by a variance of polygenic effect and kinship matrix (a matrix of kinship coefficients, which are measures of degrees of genetic correlations between individuals). Linear mixed models have been commonly used in single-marker GWASs for family data [27, 28]. Recently, SKAT has been extended to be applicable for quantitative traits in family samples [29–31]. Furthermore, the extension to dichotomous traits in family samples is described in Ionita-Laza et al. [32], in which the algorithm follows a generalized linear model and incorporates laws of Mendelian transmission to calculate the genotype expectation conditional on parental genotypes. This method, however, ignores parental phenotypes and, therefore, may lose power because it does not fully use the data. In addition, this method cannot handle nonparental rela-

tionships such as those between offspring, grandparents-grandchildren, or uncles-nephews.

In this study, we aim to propose a method that can handle dichotomous traits in family samples. Our proposed method uses a kernel machine regression and can be viewed as a generalization of famSKAT [29]. Our new model, denoted as F-SKAT, is based on a generalized linear mixed-model framework that is more general and can be applied to a larger range of studies with different types of traits. We demonstrate in our simulation studies that the original SKAT has inflated type I error rates when applied to all family samples without consideration of their relationship. By contrast, our proposed F-SKAT has a correct type I error rate. Moreover, because it uses all family samples, F-SKAT is consistently more powerful than SKAT with the use of only unrelated individuals (founders) in the family data because SKAT can only use a subset of the samples for analysis in order to retain the correct type I error rate. This is also consistent with the simulation results for quantitative traits in Chen et al. [29]. The same observation was also made in other studies [33].

Methods

SKAT in a Generalized Linear Mixed-Model Framework

The model setup is presented in a manner very similar to that of Chen et al. [29], although for noncontinuous traits instead. We assume that the $n \times 1$ vector of trait y follows a generalized linear mixed model. Now, the trait is no longer assumed to follow a normal distribution. The link function $h(\cdot)$ is used to map a linear combination of predictors for observation i , η_i , to the conditional mean of observation i , $\mu_i = E(y_i | u, \gamma)$, shown as

$$h(\mu) = \eta = X\beta + G\gamma + Zu,$$

where X is an $n \times p$ covariate matrix, β is a $p \times 1$ vector standing for fixed effects parameters (an intercept and $p - 1$ covariates), G is an $n \times q$ genotype matrix for q genetic variants of interest, γ is a $q \times 1$ vector for the random effects of variants, Z is an $n \times k$ matrix for k random effects, and u is a $k \times 1$ vector for the random effect coefficients, which is added to the original SKAT model [9, 21]. The random effect γ_j is assumed to be normally distributed with mean 0 and variance τW_j for variant j , so the null hypothesis being tested is $H_0: \gamma = 0$ that is equivalent to testing $H_0: \tau = 0$, which can be tested with a variance component score test [21] in the mixed model. Also assumed is that u is normally distributed and uncorrelated with γ , as in

$$\begin{aligned}\gamma &\sim N(0, \tau W), \\ u &\sim N(0, K),\end{aligned}$$

where W is a predefined $q \times q$ diagonal weight matrix for each variant and may use $\sqrt{w_i} = \text{Beta}(\text{MAF}_i, 1, 25)$ as in SKAT [21], and K is a $k \times k$ covariance matrix.

Following the same rationale as in the derivation of the SKAT and famSKAT score statistics [29, 34–36] (see online suppl. mate-

rial for details, www.karger.com/doi/10.1159/000375409), we have the following test statistic for our new model

$$Q = (y^* - X\hat{\beta})' \hat{\Sigma}^{-1} G W G' \hat{\Sigma}^{-1} (y^* - X\hat{\beta}),$$

where y^* is the final working trait vector, $\hat{\beta}$ is the vector of estimated fixed effects of covariates under H_0 , and $\hat{\Sigma}$ is the estimated variance-covariance matrix under H_0 . The statistic Q is a quadratic form of $(y^* - X\hat{\beta})$ and follows a mixture of χ^2 distributions [37] under H_0 . The p values can be calculated by numerical algorithms such as the Davies method [38]. This generalized linear model framework is very general with many models as special cases depending on the data type of the phenotype. For example, this framework can be simplified for continuous and dichotomous traits in a population-based study (see online suppl. Appendix), which is the same as the models described in Wu et al. [21]. In addition, count traits can also be handled. Furthermore, longitudinal and family structures can be added in the model by manipulating the random effect term.

SKAT for Dichotomous Traits in Family Samples

Specifically, the above approach can be used for handling dichotomous traits in family samples such that $h(\cdot)$ is replaced with $\text{logit}(\cdot)$ and Z_u is replaced with δ that is an $n \times 1$ vector for the random effects of family correlation. The dichotomous trait y follows a generalized linear mixed model

$$\text{logit}(P(y = 1)) = \text{logit}(\mu) = X\beta + G\gamma + \delta,$$

where $X\beta$ and $G\gamma$ are the same as defined above. Again, the random effect vector γ is assumed to follow a normal distribution with mean 0 and covariance matrix τW , so $H_0: \tau = 0$, where W is the predefined weight matrix and may use $\sqrt{w_i} = \text{Beta}(\text{MAF}_i, 1, 25)$. In addition, δ follows a normal distribution with mean 0 and covariance matrix $\sigma_\delta^2 \Phi$, where Φ is twice the $n \times n$ kinship matrix obtained from family information only.

Simulation Study

We simulated samples based on a collection of 10,000 haplotypes over a 200-kb region generated by the calibrated coalescent model [39] with mimicked linkage disequilibrium structure of European ancestry. Almost all of the simulated variants are rare variants; 1,200 haplotypes were randomly selected as parents' haplotypes. The offspring haplotypes were generated by randomly transmitting 1 of the 2 haplotypes of the father and the mother. We generated 300 families with a father, mother, and at least 1 offspring in each family for a scenario of families with flexible numbers of offspring (sibship size range: 1–5; fig. 1a). For simplicity, we also considered the scenario of family trios by selecting a father, mother, and 1 offspring from the 300 families (fig. 1b). In addition, in order to simulate a complex pedigree, we randomly selected 400 haplotypes as grandparents' haplotypes and 400 haplotypes as independent parents' haplotypes. For the 3-generation family scenario, we generated 100 families with 2 grandparents, 2 independent parents, 2 dependent parents who are the offspring of the 2 grandparents, and 4 children (fig. 1c). Furthermore, 30 randomly selected SNPs with at least one copy of a minor allele appearing in the corresponding data set were used in the analysis. The genotype can be easily converted from the haplotype data, and we simulated 100 such genotype data sets in the analysis for each of the three scenarios.

Fig. 1. Three scenarios of pedigree structures in simulation studies. **a** Families with flexible numbers of offspring. **b** Family trios. **c** Families with 3 generations.

Type I Error Rate

In analyzing the scenarios of families with flexible numbers of offspring and families with 3 generations, we compared F-SKAT to 3 other approaches: (1) the approach that applies SKAT on unrelated individuals (founders) in the family data (abbreviated as unrSKAT), (2) the original SKAT, and (3) the method by Ionita-Laza et al. (abbreviated as IL) [32]. For each of the 100 genotype data sets, we simulated 1,000 sets of phenotypes. The dichotomous phenotypes for each family were generated via the following model

$$\text{logit}(P(y = 1)) = \alpha_0 + \delta,$$

where α_0 was determined to set the prevalence to 10% in our simulation. In other words, $\alpha_0 = \log(0.1/0.9)$, and δ is from a multivariate normal distribution with means 0 and covariance $\Sigma = \sigma_\delta^2 \Phi$, where Φ is twice the kinship matrix. For instance, a family with a father, mother, and 2 children has

$$\Sigma_i = \sigma_\delta^2 \begin{bmatrix} 1 & 0 & 0.5 & 0.5 \\ 0 & 1 & 0.5 & 0.5 \\ 0.5 & 0.5 & 1 & 0.5 \\ 0.5 & 0.5 & 0.5 & 1 \end{bmatrix},$$

and $\sigma_\delta^2 = 5$. The scenario of families with 3 generations has a more complicated kinship matrix (online suppl. fig. 1S). The phenotypes for all of the families were generated in the same way, and the 1,000 simulated phenotypes for each of the 100 genotype data sets were used to evaluate the type I error rate. For the scenario of family trios, both parents and 1 child were selected from each of the families with flexible numbers of offspring. For these families, the covariance matrix Σ_i is the same as for all the families, where

$$\Sigma_i = \sigma_\delta^2 \begin{bmatrix} 1 & 0 & 0.5 \\ 0 & 1 & 0.5 \\ 0.5 & 0.5 & 1 \end{bmatrix}.$$

Power Evaluation

We used the same genotype data sets as described above. We compared F-SKAT with unrSKAT and IL for the scenarios of families with flexible numbers of offspring and families with 3 generations. For each of the 100 genotype data sets, we simulated 1,000

Table 1. Type I error rate of F-SKAT, unrSKAT, SKAT, and IL for parent-offspring family trios

	$\alpha = 0.05$	$\alpha = 0.01$	$\alpha = 0.005$	$\alpha = 0.001$
F-SKAT	0.05188	0.01109	0.00577	0.00131
unrSKAT	0.04887	0.01009	0.00535	0.00138
SKAT	0.08802	0.02254	0.01239	0.0035
IL	0.05528	0.01380	0.00789	0.00195

Table 2. Type I error rate of F-SKAT, unrSKAT, SKAT, and IL for families with flexible numbers of offspring

	$\alpha = 0.05$	$\alpha = 0.01$	$\alpha = 0.005$	$\alpha = 0.001$
F-SKAT	0.05421	0.01151	0.00589	0.00134
unrSKAT	0.04887	0.01009	0.00535	0.00138
SKAT	0.12263	0.03530	0.02047	0.00598
IL	0.05242	0.01350	0.00766	0.00235

Table 3. Type I error rate of F-SKAT, unrSKAT, SKAT, and IL for families with 3 generations

	$\alpha = 0.05$	$\alpha = 0.01$	$\alpha = 0.005$	$\alpha = 0.001$
F-SKAT	0.05356	0.01201	0.00625	0.00158
unrSKAT	0.04779	0.00989	0.00527	0.00142
SKAT	0.17455	0.05511	0.03308	0.01022
IL	0.05159	0.01269	0.00727	0.00189

sets of phenotypes. The dichotomous phenotypes for each family were simulated via the following model

$$\text{logit}(P(y_i = 1)) = \alpha_0 + 0.5X_{1i} + 0.5X_{2i} + \beta_1 G_{1i} + \beta_2 G_{2i} + \dots + \beta_k G_{ki} + \delta_i,$$

where X_{1i} is a continuous variable generated from a standard normal distribution, X_{2i} is a dichotomous variable from a Bernoulli distribution with a probability of 0.5, G_{1i} , G_{2i} , ..., G_{ki} are the genotypes of causal SNPs, and β_1 , β_2 , ..., β_k are log odds ratios of the causal SNPs. We considered that 40% and 80% of all variants are disease susceptibility variants and that α_0 and δ_i were determined the same way as described in the Type I Error Rate section. Furthermore, β_1 , β_2 , ..., β_k were set to $c \log_{10} \text{MAF}_i$ in order to assign large weights to rare variants, where $c = 0.4$ is chosen such that when $\text{MAF} = 0.0001$, $\beta = 1.6$ (i.e., odds ratio = 4.9) [21]. Because SKAT can handle the presence of both risk and protective variants, we also considered that 25% of the causal variants are protective, which means $\beta = -c \log_{10} \text{MAF}_i$ (i.e., 30% disease variants and 10% protective variants; and 60% disease variants and 20% protective variants). The phenotypes for all of the families were generated the same way, and the 1,000 phenotypes for each of the 100 genotype

Fig. 2. QQ plot of the p values for F-SKAT, unrSKAT, SKAT, and IL for parent-offspring family trios from the null simulation, with a 95% pointwise confidence band (gray area) that is computed under the assumption of the p values being drawn independently from a uniform [0, 1] distribution.

data sets were used to evaluate the power. For the scenario of family trios, the simulated phenotypes of father, mother, and 1 offspring were selected from each of the families with flexible numbers of offspring.

Results

Simulation of the Type I Error Rate

Tables 1–3 depict the empirical type I error rates of F-SKAT, unrSKAT, SKAT, and IL at α levels of 0.05, 0.01, 0.005, and 0.001 for family trios, families with flexible numbers of offspring, and families with 3 generations. The results indicate that the type I error is inflated when SKAT is applied directly to all of the samples including correlated individuals. In contrast, F-SKAT, unrSKAT, and IL retain the correct type I error rate, but IL shows a trend of increased type I error rate as the significance level decreases. From the quantile-quantile (QQ) plots in figures 2 and 3, we can see similar patterns. This indicates that F-SKAT and unrSKAT can control type I error well for different significance levels; however, IL maintains the correct type I error rate when the significance level is not stringent (say, $\alpha > 0.005$) but otherwise has an inflated type I error rate. The QQ plot in figure 4 indicates that

Fig. 3. QQ plot of the p values for F-SKAT, unrSKAT, SKAT, and IL for families with flexible numbers of offspring from the null simulation, with a 95% pointwise confidence band (gray area) that is computed under the assumption of the p values being drawn independently from a uniform [0, 1] distribution.

Fig. 4. QQ plot of the p values for F-SKAT, unrSKAT, SKAT, and IL for families with 3 generations from the null simulation, with a 95% pointwise confidence band (gray area) that is computed under the assumption of the p values being drawn independently from a uniform [0, 1] distribution.

F-SKAT preserves the desired type I error rate, and unrSKAT and IL maintain the correct type I error rate at nonstringent significance levels. The inflation of the type I error rate in SKAT becomes more severe as the number of correlated individuals in one family increases.

Statistical Power Comparison

Because the original SKAT has an incorrect type I error rate for family data, we only investigated the power of F-SKAT, unrSKAT, and IL (i.e. exclude the original SKAT from the power comparison). The simulation results for family trios, families with flexible numbers of offspring, and families with 3 generations are shown in figures 5–7, respectively. For family trios, in all of the scenarios we considered, the power of F-SKAT is consistently but only slightly higher than that of unrSKAT. The slight gain in power may be caused by the difference in type I error rate (online suppl. fig. 2S). The power of IL is not high in our simulation study. For families with flexible numbers of offspring and families with 3 generations, the pattern is similar to that of the family trios. In all scenarios, the power of F-SKAT is consistently higher than that of unrSKAT and IL. Furthermore, F-SKAT achieves the highest power in families with 3 generations, and the power of F-SKAT

is higher in families with flexible numbers of offspring than in family trios, which indicates that the power gain of F-SKAT increases as the size of families increases. This is expected because F-SKAT makes full use of the data, while in contrast, unrSKAT uses only unrelated samples and discards other family members.

Discussion

In this paper, we have proposed a new method, F-SKAT, under a generalized linear mixed-model framework that can be used to analyze family data with various phenotypes, such as continuous and discrete, and covariates. The new method is based on a kernel machine regression and can be viewed as an extension of SKAT [9, 21] as well as famSKAT [29]. As a set-based analysis, F-SKAT shares the advantages of set-based methods, such as improved power by testing a set of genetic variants jointly and by reducing the multiple-testing penalty. Our simulation studies show that the proposed method has consistently higher power than existing approaches in the scenarios we have considered. The new method includes various existing methods, such as SKAT and famSKAT,

Fig. 5. Power comparisons of F-SKAT, unrSKAT, and IL for data with parent-offspring family trios (α level ranges from 0 to 0.05). **a** 40% disease variants scenario; **b** 80% disease variants scenario; **c** 30% disease variants and 10% protective variants scenario; **d** 60% disease variants and 20% protective variants scenario.

Fig. 6. Power comparisons of F-SKAT, unrSKAT, and IL for data of families with flexible numbers of offspring (α level ranges from 0 to 0.05). **a** 40% disease variants scenario; **b** 80% disease variants scenario; **c** 30% disease variants and 10% protective variants scenario; **d** 60% disease variants and 20% protective variants scenario.

as special cases. This shows that the proposed method is theoretically more advantageous than the existing methods and allows us to conveniently analyze data using different approaches.

In the simulation studies, we show that using SKAT on data with related samples results in an inflated type I error rate, which is consistent with the results for quantitative traits in Chen et al. [29]. One strategy is to analyze only

Fig. 7. Power comparisons of F-SKAT, unrSKAT, and IL for data of families with 3 generations (α level ranges from 0 to 0.05). **a** 40% disease variants scenario; **b** 80% disease variants scenario; **c** 30% disease variants and 10% protective variants scenario; **d** 60% disease variants and 20% protective variants scenario.

unrelated subjects from the data using SKAT. In this way, the type I error rate can be controlled, but power is sacrificed because only part of the data is analyzed. In contrast, F-SKAT uses all of the data, retains the correct type I error rate, and achieves higher power in all of the scenarios we considered. Our simulation also shows that the larger the family size, the more power F-SKAT gains, which is also consistent with the findings for quantitative traits in Chen et al. [29]. Based on our simulation study, F-SKAT makes a good choice for analysis of family data with various traits, although we only considered dichotomous traits in this study. For quantitative traits, an extensive simulation study has been published by Chen et al. [29].

The computation time of F-SKAT depends on both sample size (including family size and structure) and the number of genetic variants to be analyzed. F-SKAT involves fitting a generalized linear mixed model that must be done iteratively. This consumes much of the computation time. Even though this step is computationally intensive, the total computation time, in fact, may not be a serious issue when analyzing data from GWASs. Like SKAT, F-SKAT is basically a score test and, thus, under H_0 , the estimates of covariate coefficients and covariance matrix are independent with genetic variants. The generalized linear mixed model under H_0 only needs to be fitted once for the whole genome and is then reused in the anal-

ysis of other genes. Therefore, the total computation time is greatly reduced. To estimate the computation time of the proposed methods, we conducted a simple simulation in R on a single computing node with a 3-GHz CPU and 4 GB memory, analyzing a 200-kb region on 1,500 individuals (500 trios). F-STAT needed 5.988 s for the analysis. Based on this simulation, we can estimate that it may take F-STAT approximately 25 h to analyze the whole genome (~ 3 Gb) on the same samples. Using a computer cluster with multiple nodes, we anticipate that most of the genome-wide data analysis should be finished within hours using the proposed methods.

Thus, complicated generalized linear mixed models can be implemented for GWASs without suffering from a huge amount of computation time. However, if the number of markers in a gene is large, inverting the large matrix is still computationally intensive. One way to handle this would be to partition the markers into smaller groups, such as groups of nonsynonymous or synonymous coding variants. Another way would be to use fast algorithms, such as those implemented in the software EMMA/EMMAX [40, 41], to make our algorithm faster and more efficient. The approach of clustering samples implemented in TASSEL [41] applies naturally to family data. Recently, several new fast algorithms have been proposed for mixed model [42–45]. Some of the new ideas

may be used in our algorithm. The F-SKAT algorithm has been implemented in R (<http://www.r-project.org/>) and the source code is available online (<http://www.soph.uab.edu/ssg/software>).

In our work, the kinship coefficients in the kinship matrices were obtained from family relationships. If genome-wide genotype data are available, it is more advantageous to use genetic markers to estimate the kinship coefficients among individuals [40, 46–50]. The use of kinship coefficients enables our method to be applicable to data with any relationship (such as grandparents-grandchildren and uncles-nephews) and even relationships with cryptic relatedness. We have shown that our new method is feasible for genome-wide studies, although the computation time is still intensive. Fast algorithms, such as those developed for linear mixed models [31, 40, 41, 43–45], are attractive and would be very helpful. Although we have only studied the performance of a linear

kernel in this work, it is straightforward to use a non-linear kernel within the flexible kernel machine-regression framework when a non-linear association between a disease and genetic variants is assumed.

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