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Maximizing the Power of Genome-Wide Association Studies: A Novel Class of Powerful Family-Based Association Tests

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Abstract

For genome-wide association studies in family-based designs, a new, universally applicable approach is proposed. Using a modified Liptak's method, we combine the p -value of the family-based association test (FBAT) statistic with the p -value for the Van Steen-statistic. The Van Steen-statistic is independent of the FBAT-statistic and utilizes information that is ignored by traditional FBAT-approaches. The new test statistic takes advantages of all available information about the genetic association, while, by virtue of its design, it achieves complete robustness against confounding due to population stratification. The approach is suitable for the analysis of almost any trait type for which FBATs are available, e.g. binary, continuous, time to-onset, multivariate, etc. The efficiency and the validity of the new approach depend on the specification of a nuisance/tuning parameter and the weight parameters in the modified Liptak's method. For different trait types and ascertainment conditions, we discuss general guidelines for the optimal specification of the tuning parameter and the weight parameters. Our simulation experiments and an application to an Alzheimer study show the validity and the efficiency of the new method, which achieves power levels that are comparable to those of population-based approaches.

Keywords

FBAT; Liptak's method; Tuning parameter

Introduction

Transmission-disequilibrium tests (TDTs) are joint genetic tests for linkage and association in family-based designs in which genetic information is available on one affected offspring and its parents. Since the invention of the first TDTs by Spielman and Ewens [33], they have played a prominent role in genetics. Their universal robustness against confounding due to population admixture made them the gold-standard yardstick to which all other statistical methods that were developed afterwards have to be compared. In applications, they have led to the identification of many novel disease genes [2,24,30–32] and confirmed/replicated numerous existing findings [12,21,27].

The universal robustness of the TDT-approach and of its generalization, the family-based association test (FBAT) [15,16], stems from the fact that the tests only utilize information about the transmissions of alleles within ones' families, the so-called *within-family information* [9]. The part of the data that contains information about the association between the genetic locus and the phenotype at the population level, *the between-family information*, is not included in such tests. As a consequence, they are not fully efficient.

With the arrival of genome-wide association studies (GWAS) in which several hundreds of thousands genetic loci are genotyped, the multiple testing problem became the major roadblock in terms of the statistical analysis. The selection of the study design and the choices for test statistics were driven by the goal of maximizing the statistical power. For family-based designs, 2-stage testing strategies [13,34] were proposed that are able to incorporate the between-family information into the analysis to increase the statistical power, while maintaining the complete robustness of the traditional TDT/FBAT approach. An alternative approach is to sacrifice the complete robustness of the TDT/FBAT approach and to analyze family-data as a population-based study by modeling the phenotype directly as a function of the genotype. The specification of the variance/covariance matrix [4,7] is used to account for the phenotypic and genetic correlation among related individuals. In such an analysis approach, one has to adjust for population admixture by using population-based analysis approaches such genomic control [5,6], STRUCTURE [26], EIGENSTRAT [25], etc. However, it is important to note that complete robustness cannot be achieved by such an approach. Furthermore, it is not clear how the covariance matrix of common environmental effects can be specified in a large pedigree with multiple generations.

Recently, we proposed a novel overall family-based association test by combining directly the between-family component and the within-family component in the analysis [36], using a modified Liptak's method [23]. By virtue of its design, the new approach remains completely robust against population admixture, while achieving the same power levels as population-based methods. In the modified Liptak's method, the robustness of the approach is ensured by the use of rank-based p -values for the between-family component. In general, the rank-based p -values for the between family component will depend on a nuisance/tuning parameter. We will discuss optimal choices for the tuning parameter that provides robustness against confounding and achieves the optimal power at the same time.

The other parameters that have to be specified in the modified Liptak's method are the weights for the between-family component and the within-family component. It was shown that the optimal weights depend on the informativity of both components when independent p -values are combined [35]. As a result they will depend on the trait type of the phenotype of interest and the ascertainment condition that was used for the recruitment of the study. We will derive optimal choices for the most common design type and phenotypes.

Using the simulation studies, we illustrate the robustness of the proposed overall test, and compare its power to other frequently used analysis approaches. The approach is illustrated by an application to a GWAS for Alzheimer.

2 Method

In a family-based association study, we assume that the members of each family for which DNA is available have been genotyped at m loci. For simplicity, we assume that only nuclear families are available, even though the proposed method can be extended to larger pedigree.

At the k th marker locus, the family-based association test is computed for the phenotype of interest and it is denoted by FBAT_k . The statistic FBAT_k is constructed based on the offspring phenotype and the within-family information. The within-family information is referred to here as the difference between the observed marker score and the expected marker score which is computed conditional upon the parental genotypes/sufficient statistic [28], assuming Mendelian transmissions. Similarly, for the k th marker, the between-family information is defined as the expected marker score conditional on the parental genotypes/sufficient. The between-family information and the offspring phenotype are used to assess the evidence for association at a population-based level by computing a Van Steen-type “screening-statistic” T_k [13,34]. Typically, the Van Steen-type statistic is computed based on the conditional mean model [18,19]. Here the phenotype of the non-founders in the pedigrees is the outcome variable that is regressed on the parental genotypes/sufficient statistic of the pedigree [28]. The statistic T_k can be either a Wald-test for the genetic effect size or the estimated power of the FBAT-statistic [34]. It can be shown very generally that both statistics are statistically independent (see [14] for the proof).

While the FBAT-statistic is constructed purely based on the within-family information, i.e. the Mendelian transmissions, and is thereby robust against confounding due to population admixture, the population-based Van Steen-type statistic T_k is vulnerable to such effects, as any population-based approach. Since the FBAT-statistic does not utilize all available information about the association, i.e. the Van Steen-type statistic, it is not optimal in terms of statistical power. In order to maximize the statistical power in family-based designs, we propose to combine here both sources of information, the FBAT-statistic FBAT_k and the screening statistic T_k , using a modified Liptak’s method that maintains the robustness of family-based designs. We let $T_{(k)}$ be the ordered statistic and $T_{(m)}$ denote the screening statistic with the least amount of evidence for association based on the information in the statistic FBAT_k , i.e. $T_{(m)} \leq T_{(m-1)} \leq \dots \leq T_{(1)}$. We then compute the rank-based p -value, $pT_{(k)}$, for $T_{(k)}$,

$$(k - \delta)/m,$$

where δ is a nuisance/tuning parameter. The parameter δ is included to control the type-1 error, i.e. to avoid that the approach becomes conservative. Let $p\text{FBAT}_k$ be the p -value of the FBAT-statistic for the k th marker, FBAT_k . If Z_p and $\Phi(\cdot)$ are the p th quantile and the cumulative normal distribution of the standard normal distribution respectively, the *overall family-based test for association* can be defined by the combining p -value of the FBAT-statistic and the one-tailed Van Steen-statistics, i.e.

$$\Phi(w_{\text{FBAT}} Z_{p\text{FBAT}_k} + w_T Z_p T_k),$$

where the parameters w_{FBAT} and w_T are standardized weights so that $w_{\text{FBAT}}Z_{p\text{FBAT}_k} + w_T Z_{pT_k}$ has a normal distribution with mean 0 and variance 1, i.e. $w_{\text{FBAT}}^2 + w_T^2 = 1$. In the literature, this approach combining two test statistics is known as the Liptak's method [23].

First, we will confirm the independence of two statistics, FBAT_k and T_k . We assume that $X_{ji,k}$ and Y_{ji} are the offspring genotype at the k th marker and phenotype for j th individual in the i th family respectively, where $j = 1, 2, \dots, n_i$ and $i = 1, 2, \dots, n$. $j = 1$ or 2 indicates the parents. $X_{ji,k}$ can be 0, 1 or 2, according to the number of minor allele. Then the log-likelihood for the nuclear family i is

$$l(Y_{1i}, \dots, Y_{n_i}, X_{1i,k}, \dots, X_{n_i,k}) = l(Y_{1i}, \dots, Y_{n_i}, X_{1i,k}, \dots, X_{2i,k}) + l(X_{3i,k}, \dots, X_{n_i,k} | X_{1i,k}, X_{2i,k}, Y_{3i}, \dots, Y_{n_i}).$$

Because the distribution of the FBAT_k statistic is defined by $l(X_{3i,k}, \dots, X_{n_i,k} | X_{1i,k}, X_{2i,k}, Y_{3i}, \dots, Y_{n_i})$ and the screening statistic T_k has the likelihood function $l(Y_{1i}, \dots, Y_{n_i} | X_{1i,k}, X_{2i,k})$, this guarantees that FBAT_k and T_k are independent.

2.1 Validity and Tuning Parameter

The rank-based p -value for the screening statistic T_k always generates the same distribution over the markers in a GWAS. On average, the proportion of the significant markers is fixed at the specified α -level. Also, if the number of markers/tests is relatively large and the number of true-positives is small as well, the rank-based p -values will be asymptotically equal to the distribution-based p -value. Obviously, for small values of δ , the rank-based p -value pT_k is conservative and, for large values of δ , anti-conservative. As a consequence, the nuisance/tuning parameter δ needs to be carefully chosen for the proposed method. Rules of thumb for its specification will be derived by assessing the genome-wide type-1 error rate for a prespecified α -level.

2.1.1 Genome-Wide Type-1 Error Rate—If we assume that $f(p\text{FBAT}_k, pT_k; w_{\text{FBAT}}, w_T)$ is the method to combine p -values $p\text{FBAT}_k$ and pT_k with weights w_{FBAT} and w_T , the genome-wide type-1 error rate at a prespecified α -level is defined as the expected number of the markers of which p -values are less than α :

$$E \left[\frac{1}{m} \sum_{k=1}^m I\{f(p\text{FBAT}_k, pT_k; w_{\text{FBAT}}, w_T) < \alpha\} \right].$$

For the Liptak's method, $f(p\text{FBAT}_k, pT_k; w_{\text{FBAT}}, w_T)$ is $\Phi(w_{\text{FBAT}}Z_{p\text{FBAT}_k} + w_T Z_{pT_k})$. Because the FBAT_k is robust to the population stratification, we can assume that $p\text{FBAT}_k$ follows uniform distribution under the null hypothesis. In the presence or absence of population admixture, the validity of the proposed method can be shown for $\delta = 0.5$ if the α -level is less than or equal to 0.5 [36]. One can show that

$$\begin{aligned} & \frac{1}{m} \sum_{k=1}^m P \left[\Phi \left(w_{\text{FBAT}} Z_{p\text{FBAT}_k} + w_T Z_{\frac{k-\delta}{m}} \right) < \alpha \right] \\ &= \frac{1}{m} \sum_{k=1}^m \Phi \left[\frac{1}{w_{\text{FBAT}}} (Z_\alpha - w_T Z_{\frac{k-\delta}{m}}) \right]. \end{aligned}$$

It should be noted that the genome-wide type-1 error rate is an increasing function of δ . If we assume $\delta = 0.5$ and let $g_x(\alpha)$ be

$$g_x(\alpha) := \Phi\left\{\frac{1}{w_{\text{FBAT}}}(Z_\alpha - w_T Z_x)\right\} + \Phi\left\{\frac{1}{w_{\text{FBAT}}}(Z_\alpha + w_T Z_x)\right\} - 2\alpha,$$

$g_x(\alpha) = 0$ guarantees that $\frac{1}{m} \sum_{k=1}^m \Phi\left[\frac{1}{w_{\text{FBAT}}}(Z_\alpha - w_T Z_{\frac{k-0.5}{m}})\right] \leq \alpha$ because $Z_{\frac{(m-k+1)-0.5}{m}} = Z_{1-\frac{k-0.5}{m}} = -Z_{\frac{k-0.5}{m}}$ and

$$\begin{aligned} & \sum_{k=1}^m \Phi\left[\frac{1}{w_{\text{FBAT}}}(Z_\alpha - w_T Z_{\frac{k-0.5}{m}})\right] - m \cdot \alpha \\ & \leq \sum_{k=1}^{\lfloor m/2 \rfloor} \left\{ \Phi\left[\frac{1}{w_{\text{FBAT}}}(Z_\alpha - w_T Z_{\frac{k-0.5}{m}})\right] + \Phi\left[\frac{1}{w_{\text{FBAT}}}(Z_\alpha - w_T Z_{\frac{m-k+1-0.5}{m}})\right] \right\} \\ & \quad - \left\lfloor \frac{m}{2} \right\rfloor \cdot 2\alpha \\ & \leq \sum_{k=1}^{\lfloor m/2 \rfloor} \left\{ \Phi\left[\frac{1}{w_{\text{FBAT}}}(Z_\alpha - w_T Z_{\frac{k-0.5}{m}})\right] + \Phi\left[\frac{1}{w_{\text{FBAT}}}(Z_\alpha + w_T Z_{\frac{k-0.5}{m}})\right] - 2\alpha \right\} \\ & \leq \sum_{k=1}^{\lfloor m/2 \rfloor} g_{\frac{k-0.5}{m}}(\alpha), \end{aligned}$$

where $\lfloor m/2 \rfloor$ means the largest integer that is less than or equal to $m/2$. Because $\frac{\partial^2}{\partial \alpha^2} g_x(\alpha) > 0$, $g_x(\alpha)$ is maximized at either $\alpha = 0$ or 0.5 for $\alpha \in [0, 0.5]$, and $g_x(0.5) = 0$ and $\lim_{\alpha \rightarrow 0} g_x(\alpha) = 0$. As a result, we can conclude that the proposed p -value is valid even under the population stratification if $\delta = 0.5$.

However, as one can verify numerically, the parameter choice $\delta = 0.5$ will be slightly conservative for the studies with large number of SNPs at the small significance level. More powerful parameter choices can be obtained by numerical approaches.

Alternatively, one could consider to combine the p -values for the FBAT-statistic and the Van Steen screening statistic by Fisher's method [8]. If we let $\chi^2_{\text{df}=4}(\cdot)$ be the cumulative χ^2 -distribution function with 4 degrees of freedom, $f(p\text{FBAT}_k, pT_k; w_{\text{FBAT}}, w_T)$ is $1 - \chi^2_{\text{df}=4}[-2(\log p\text{FBAT}_k + \log pT_k)]$ for Fisher's method. When $p \sim U(0, 1)$, $-2 \log p$ follows $\chi^2_{\text{df}=2}$ and it is why we have $\chi^2_{\text{df}=4}$. One can show that the type-1 error rate is

$$\begin{aligned} & E \left[\frac{1}{m} I \left\{ \left(1 - \chi^2_{\text{df}=4}(-2 \log p\text{FBAT}_k - 2 \log pT_k) \right) < \alpha \right\} \right] \\ & = \sum_{k=1}^m \exp \left[-\log \frac{k-\delta}{m} - 0.5 \chi^2_{\text{df}=4}(1-\alpha) \right]. \end{aligned}$$

2.1.2 Family-Wise Error Rate—The family-wise type-1 error at the genome-wide significance level α means the probability of making one or more false discoveries. If we use Bonferroni-correction, then genome-wide type-1 error is α/m , and under the null hypothesis the family-wise error rate for the proposed method is

$$\begin{aligned} & P \left[\bigcup_{k=1}^m \left\{ \Phi(\mathbf{w}_{\text{FBAT}} \mathbf{Z}_{\text{pFBAT}_k} + \mathbf{w}_T \mathbf{Z}_{pT_k}) < \frac{\alpha}{m} \right\} \right] \\ & \leq \sum_{k=1}^m P \left[\left\{ \Phi(\mathbf{w}_{\text{FBAT}} \mathbf{Z}_{\text{pFBAT}_k} + \mathbf{w}_T \mathbf{Z}_{\frac{k-\delta}{m}}) < \frac{\alpha}{m} \right\} \right] \\ & = \sum_{k=1}^m \Phi \left[\frac{1}{w_{\text{FBAT}}} (Z_{\frac{\alpha}{m}} - w_T Z_{\frac{k-\delta}{m}}) \right]. \end{aligned}$$

In the same way, we can have for the Fisher's method:

$$\begin{aligned} & P \left[\bigcup_{k=1}^m \left\{ \left(1 - \chi_{\text{df}=4}^2 (-2 \log \mathbf{p}_{\text{FBAT}_k} - 2 \log pT_k) \right) < \frac{\alpha}{m} \right\} \right] \\ & = \sum_{k=1}^m \exp \left[-\log \frac{k-\delta}{m} - 0.5 \chi_{\text{df}=4}^{-2} \left(1 - \frac{\alpha}{m} \right) \right]. \end{aligned}$$

These results show that the family-wise error rates are less than or equal to the genome-wide type-1 error rates. As a result, both the family-wise error rate and genome-wide type-1 error rate will be very similar for the proposed overall family-based association test.

2.2 Optimal Weights

It was shown [35] that optimal weights are one of the most important factors when independent p -values are combined. The optimal weights are the ratios of the expectation of the statistics under alternative hypothesis [35]. Even though it is hard in general to specify the alternative hypothesis and to compute the expected value of the test statistics, family-based association studies are special cases in which this is possible for some of the most important phenotypes/designs.

2.2.1 Random Samples—The standard genetic model is usually assumed as

$$E(Y_{ji}|X_{ji,k}) = \beta_0 + \beta_1 \cdot X_{ji,k}.$$

Since the direct use of the above marker scores would bias the nominal significance level of FBAT_k , we consider the following conditional mean model for T_k :

$$E(Y_{ji}|X_{1i,k}, X_{2i,k}) = \begin{cases} \beta_0 + \beta_1 \cdot E(X_{ji,k}|X_{1i,k}, X_{2i,k}) & \text{if } j > 2, \\ \beta_0 + \beta_1 X_{ji,k} & \text{if } j \leq 2. \end{cases}$$

Then, as long as the unbiased estimator for β is used, our statistic, T_k , for between-family component is given by

$$T_k = \frac{\widehat{\beta}_1}{\sqrt{\text{var}_{Y|X_1, X_2}(\widehat{\beta}_1)}}$$

and

$$w_T = \frac{E_{Y|X_1, X_2}(\hat{\beta}_1)}{\sqrt{\text{var}_{Y|X_1, X_2}(\hat{\beta}_1)}} = \frac{\beta_1}{\sqrt{\text{var}_{Y|X_1, X_2}(\hat{\beta}_1)}}.$$

$\hat{\beta}_1$ and $\text{var}_{Y|X_1, X_2}(\hat{\beta}_1)$ can be estimated with the linear mixed model under the conditional mean model.

For within-family component, FBAT_k is calculated conditional on the parental genotypes and the offspring phenotypes as

$$\text{FBAT}_k = \frac{\sum_{j>2,i} [X_{ji,k} - E(X_{ji,k}|X_{1i,k}, X_{2i,k})][Y_{ji} - \eta_Y]}{\sqrt{\sum_{j>2,i} [\text{var}(X_{ji,k}|X_{1i,k}, X_{2i,k})(Y_{ji} - \eta_Y)^2]}},$$

where η_Y is the offset [17], and we have

$$\begin{aligned} w_{\text{FBAT}} &= \frac{E_{Y|X}(\sum_{j>2,i} [X_{ji,k} - E(X_{ji,k}|X_{1i,k}, X_{2i,k})][Y_{ji} - \eta_Y])}{\sqrt{\sum_{j>2,i} [\text{var}(X_{ji,k}|X_{1i,k}, X_{2i,k})(Y_{ji} - \eta_Y)^2]}} \\ &= \beta_1 \frac{\sum_{j>2,i} X_{ji,k} [X_{ji,k} - E(X_{ji,k}|X_{1i,k}, X_{2i,k})]}{\sqrt{\sum_{j>2,i} [\text{var}(X_{ji,k}|X_{1i,k}, X_{2i,k})(Y_{ji} - \eta_Y)^2]}}. \end{aligned}$$

As a result, the ratio of the optimal weights is

$$w_{\text{FBAT}} : w_T = \frac{\sum_{j>2,i} X_{ji,k} [X_{ji,k} - E(X_{ji,k}|X_{1i,k}, X_{2i,k})]}{\sqrt{\sum_{j>2,i} [\text{var}(X_{ji,k}|X_{1i,k}, X_{2i,k})(Y_{ji} - \eta_Y)^2]}} : \frac{1}{\sqrt{\text{var}_{Y|X_1, X_2}(\hat{\beta}_1)}}.$$

In particular, $\sum_{j,i} X_{ji,k} [X_{ji,k} - E(X_{ji,k}|X_{1i,k}, X_{2i,k})]$ is asymptotically independent of $\sum_{j,i} [X_{ji,k} - E(X_{ji,k}|X_{1i,k}, X_{2i,k})][Y_{ji} - \eta_Y]$ and the substitution of the estimated variance of the statistics to weights does not violate the type-1 error if it is relatively large. Also, for trio designs with unknown parental phenotypes, our numerical results (Table 1) show that the optimal weights are approximately equal if the genetic effect is small and there is no ascertained sample. As a result, we recommend the use of equal weights for the trio designs.

2.2.2 Affected-Only Trio Design—The power of family-based design in which only affected offsprings have been recruited, e.g. childhood asthma management program [3], can often be improved integrating genome-wide data on unrelated controls into this analysis. As more GWAS data are produced, their genotypes, for example dbGap, become often publicly available. Such genotypes can be utilized in family-based design in which only affected offsprings have been recruited. The genotype information on the unrelated controls can be utilized for more efficient estimates of the between-family component in the analysis. We assume that there are n trios with affected offspring and n_{pop} independent control samples from the same population, and $Z_{l,k}$ indicates the genotype of l th individual in the sample of unrelated controls ($l = 1, 2, \dots, n_{\text{pop}}$). Furthermore, the minor allele frequencies in the

population and cases are denoted as p_k and p_k^a at marker locus k respectively. Again, FBAT_k for within-family component does not include the information on the unrelated controls and is given by

$$\text{FBAT}_k = \frac{\sum_i [X_{3i,k} - E(X_{3i,k} | X_{1i,k}, X_{2i,k})]}{\sqrt{\sum_i \text{var}(X_{3i,k} | X_{1i,k}, X_{2i,k})}}.$$

For T_k , we can construct a modified Cochran–Armitage (CA) test based on the between-family information for the affected offspring, i.e. their conditional genotypes, and the actual genotypes of the unrelated control sample. If we let $Y_{ji} = 1$ for affected individual and define $p_{xy,k}^a$ and $p_{\text{bet},k}^a$ as

$$\begin{aligned} p_{xy,k}^a &:= P(X_{1i,k}=x, X_{2i,k}=y \text{ or } X_{1i,k}=y, X_{2i,k}=x | Y_{3i}=1), \\ p_{\text{bet},k}^a &:= \frac{1}{2} E(E(X_{3i,k} | X_{1i,k}, X_{2i,k}) | Y_{3i}=1) = \frac{1}{4} p_{01,k}^a + \frac{1}{2} p_{02,k}^a \\ &\quad + \frac{1}{2} p_{11,k}^a + \frac{3}{4} p_{12,k}^a + p_{22,k}^a, \end{aligned}$$

then we can have the following T_k :

$$T_k = \frac{\widehat{p_{\text{bet},k}^a} - \widehat{p_{\text{pop},k}}}{\sqrt{\text{var}(\widehat{p_{\text{bet},k}^a} - \widehat{p_{\text{pop},k}})}},$$

where $\widehat{p_{\text{pop},k}}$ is the estimated minor allele frequency from the random samples. Obviously $\widehat{p_{\text{bet},k}^a}$ and $\widehat{p_{\text{pop},k}}$ can be estimated as

$$\begin{aligned} \widehat{p_{\text{bet},k}^a} &= \frac{1}{4} \widehat{p_{01,k}^a} + \frac{1}{2} \widehat{p_{02,k}^a} + \frac{1}{2} \widehat{p_{11,k}^a} + \frac{3}{4} \widehat{p_{12,k}^a} + \widehat{p_{22,k}^a}, \\ \widehat{p_{\text{pop},k}} &= \frac{1}{n_{\text{pop}}} \sum_{l=1}^{n_{\text{pop}}} \left\{ \frac{1}{2} \mathbf{I}(\mathbf{Z}_{l,k}=1) + \mathbf{I}(\mathbf{Z}_{l,k}=2) \right\}, \end{aligned}$$

where in trios with affected-only offspring

$$\widehat{p_{xy,k}^a} = \begin{cases} \frac{1}{n} \sum_{i=1}^n \{I(X_{1i,k}=x, X_{2i,k}=y) + I(X_{1i,k}=y, X_{2i,k}=x)\} & \text{if } x \neq y, \\ \frac{1}{n} \sum_{i=1}^n I(X_{1i,k}=x, X_{2i,k}=x) & \text{if } x=y. \end{cases}$$

In particular, it was shown that $\text{var}(\widehat{p_{\text{pop},k}}) = p_k(1-p_k)/(2n)$ under Hardy–Weinberg equilibrium (HWE) and $\text{var}(\widehat{p_{\text{bet},k}^a})$ can be derived with δ -method under the null hypothesis as

$$\begin{aligned} \text{var}(\widehat{p_{\text{bet},k}^a}) &= \frac{1}{8n} (p_{1,k} - p_{1,k}^2 - 4p_{1,k}p_{2,k} + 4(1-p_{2,k})p_{2,k}) \\ &= \frac{1}{4n} p_k(1-p_k) \quad \text{under HWE,} \end{aligned}$$

where $p_{1,k}$ and $p_{2,k}$ are genotype frequencies with $X_{ji} = 1$ and 2 respectively at locus k . As a result, the optimal weight for FBAT_k is

$$\begin{aligned} w_{\text{FBAT}} &= \frac{E_{X|Y=1}[\sum_i \{X_{3i,k} - E(X_{3i,k}|X_{1i,k}, X_{2i,k})\}]}{\sqrt{\sum_i \text{var}(X_{3i,k}|X_{1i,k}, X_{2i,k})}} \\ &= \frac{\eta[2p_k'' - 2p_{\text{bet},k}'']}{\sqrt{\sum_i \text{var}(X_{3i,k}|X_{1i,k}, X_{2i,k})}}. \end{aligned}$$

Also, because $Z_{L,k}$ are from random samples, the optimal weight for T_k is

$$w_T = \frac{E(\widehat{p_{\text{bet},k}^a} - \widehat{p_{\text{pop},k}})}{\sqrt{\text{var}(\widehat{p_{\text{bet},k}^a} - \widehat{p_k})}} = \frac{p_{\text{bet},k}^a - p_k}{\sqrt{\text{var}(\widehat{p_{\text{bet},k}^a} - \widehat{p_k})}}.$$

If we assume HWE in the population and $f_x := P(Y_{ji} = 1|X_{ji,k} = x)$, we can have

$$\begin{aligned} p_k^a - p_{\text{bet},k}^a &= \frac{1}{2}p_k \left\{ -(1-p_k)^2 f_0 + (1-p_k)(1-2p_k)f_1 + (1-p_k)p_k f_2 \right\}, \\ p_{\text{bet},k}^a - p_k &= \frac{1}{2}p_k \left\{ -(1-p_k)^2 f_0 + (1-p_k)(1-2p_k)f_1 + (1-p_k)p_k f_2 \right\}. \end{aligned}$$

As a result, the optimal weights for FBAT_k and T_k are

$$w_{\text{FBAT}} : w_T = \frac{2n}{\sqrt{\sum_i \text{var}(X_{3i,k}|X_{1i,k}, X_{2i,k})}} : \frac{1}{\sqrt{\text{var}(\widehat{p_{\text{bet},k}^a} - \widehat{p_k})}}.$$

Table 2 shows the empirical results for the optimal weights when the disease prevalence is 0.1 and n is 1,000, and the results show that FBAT_k is usually more informative than T_k . The results confirm that the optimal weights highly depend on the ratio of the numbers (n , n_{pop}) of trios and unrelated controls, and on the mode of inheritance. However, the disease allele frequency and the magnitude of the genetic effect size, f_2 , seem to be negligible.

3 Results

3.1 Type-1 Error and Tuning Parameter

For different sets of weights, Fig. 1 shows the contour plot for the type-1 error rate as a

function of δ and the weight ratio $\frac{w_T}{w_{\text{FBAT}}}$ for a given α -level. Each contour indicates the maximum of theoretical type-1 error rates which are numerically calculated for GWAS studies with 10,000 SNPs, 45,000 SNPs, and 500,000 SNPs at a genome-wide significance level 0.05. X -axis and Y -axis indicate the tuning parameter δ and the logarithm of the ratio w_T/w_{FBAT} with base 10, respectively. The vertical cross-section panel (to the right of the plot) shows the color legend for the calculated type-1 error rates. As a general rule of thumb, Fig. 1 shows that setting the tuning parameter δ equal to 0.7 will preserve the overall α -level if the weight ratio w_T/w_{FBAT} is 1 or greater. Also, even though a choice of 0.5 for the tuning parameter δ always ensures that the specified type-1 error is maintained, this choice will be conservative for many scenarios. The inflation of the type-1 error for a parameter choice of

0.7 is always negligible (less than 0.0004). We therefore recommend in general to specify the tuning parameter δ to be 0.7.

Table 3 shows the empirical type-1 error rates for GWAS with m markers, based on 10,000 replicates at genome-wide significance level 0.1. The offspring phenotype, Y_{ji} , is simulated from a normal distribution with mean aX_{ji} and variance 1, i.e. $\mathcal{N}(aX_{ji}, 1)$, where the parameter, a , represents the additive genetic effect size. Under the null-hypothesis of no association, a will be set to 0. Assuming HWE, the parental genotypes are generated by drawing from Bernoulli distributions defined by the allele frequencies. The marker allele frequencies are randomly sampled from a uniform distribution, $U(0.1, 0.5)$. The offspring genotypes are obtained by simulated Mendelian transmissions from the parents to the offspring. We assume here the absence of ascertainment conditions, i.e. random population sample.

For simplicity, we assume that 1,000 trios are genotyped and the parental phenotypes are unknown. We assume that we control for multiple comparison, using the Bonferroni-correction. This means that the individual significance level for each SNP is α/m , where m denotes the number of markers. Our results confirm that the tuning parameter choice of $\delta = 0.7$ preserves the α -level on average for the proposed weights.

Based on 2,000 replicates, Table 4 shows the empirical type-1 error rates for GWAS studies with 500,000 SNPs either under the absence of population admixture or in the presence of population admixture. Population stratification is here measured by the Wright's F_{ST} where $F_{ST} = 0.00$ indicates no population stratification. Values of $F_{ST} = 0.05, 0.1, 0.2$, and 0.3 reflect modest to substantial degrees of population substructure. For scenarios in which population admixture is present, we assume that the admixture is created by the presence of two subpopulations whose phenotypic means differ by 0.2. The allele frequencies for each marker in the two sub-populations are generated by the Balding–Nichols model. That is, for each marker, the allele frequency, p_k , in an ancestral population is generated from a uniform distribution between 0.1 and 0.9, $U(0.1, 0.9)$. Then, the marker allele frequencies for the two subpopulations are independently sampled from the beta distributions $(p_k(1 - F_{ST})/F_{ST}, (1 - p_k)(1 - F_{ST})/F_{ST})$. Each trio is assigned to the one of the two subpopulations with 50% probability. Over the 500,000 SNPs, the minimum of the combined p -values is calculated with $\delta = 0.7$. The proportion of SNP whose p -values are less than $0.05/500,000$ are recorded. We consider three different fixed weights ($w_{FBAT}/w_T = 2, 1$ and $1/2$) and the proposed estimated weights (LIP²) for the weight choices in the Liptak's method. Table 4 does not provide any evidence for a departure of the empirical significance levels from their theoretical values, both in the absence and presence of population substructure. This confirms our guideline recommendation that using $\delta = 0.7$ maintains correct type-1 error.

3.2 Empirical Power and Optimal Weights

For the analysis of quantitative traits, Table 5 provides the empirical power at $\alpha = 0.01$ for 500K GWAS from 50,000 replicates when there is no population stratification. To alleviate the computational intensity, T_k for 500K markers are generated under null hypothesis and they are used to get the rank for pT_k in 50,000 replicates under alternative hypothesis. Under the assumption of an additive disease model for a quantitative trait, the genetic effect, a , is given as a function of the heritability, h^2 , the disease allele frequency, p_D , and the phenotypic variance, σ^2 , by

$$a = \sigma \sqrt{\frac{1}{2p_D(1 - p_D)} \frac{h^2}{1 - h^2}}.$$

In the simulation study, we assume that $h^2 = 0.005, 0.1$ and $p_D = 0.1, 0.2$ and 0.3 . 500 and 1,000 trios are generated and the phenotypic variance is 1. In Table 5, POP indicates the use of population-based analysis, i.e. a linear regression using the true disease model. LIP^f and LIP^p indicate the method of combining the p -values p_{FBAT_k} and p_{T_k} . LIP^f is the Liptak's method using equal weights and LIP^p uses the proposed weight. The results show that LIP^f and LIP^p have the same power, which indicates that informativity of within- and between-family components is actually equal. Also, POP is the most powerful and it is followed by LIP^f and LIP^p . The power differences between population-based approach and the family-based approach (LIP^f and LIP^p) are generally small. However, it should be reminded that POP is sensitive to population stratification, while the proposed method remains always valid.

Also, we consider the empirical power for the affected-only trio design with independent population-based samples. 1,000 trios with affected offsprings are generated with either 500 or 1,000 unrelated controls. For CA test, we consider 1,000 cases and 1,000 controls, or 1,500 cases and 1,500 controls. As a result, the proposed approach uses n_{pop} (or $n_{pop} + 1,000$) more individuals with genotypes while only 1,000 individuals' phenotypes are used. For the homozygous disease genotype, the relative risk, f_2/f_0 , is assumed to be 1.1, 1.2, ... and 1.5, and the heterozygous disease genotype relative risk is obtained under additive disease mode of inheritance. The disease prevalence is assumed to be 0.1 and disease allele frequencies are 0.1 (Table 6) or 0.2 (Table 7). The rank for p_{T_k} is calculated in the same way as in Table 5 and the empirical power is estimated based on 50,000 replicates. We also provide the empirical power for Cochran–Armitage test with cases/controls (CA_s) or cases/unselected samples (CA_u). Table 6 and Table 7 show that the family-approach using the LIP^p -method is better than the family-based analysis using the LIP^f . Also, the results show that CA_s is the most powerful, followed by CA_u ; but they are sensitive to population stratification. These results suggest that family-based method can have a distinct advantage over population-based methods in this context. Furthermore, for trio-designs with affected offspring, the use of estimated weights in the Liptak's method seems to provide higher statistical power than the LIP^f -method.

3.3 Complex Designs and Ascertainment Conditions: Sensitivity Analysis of the Optimal Weight-Choices Based on a 500K GWA Study for Alzheimer's Disease

Late-onset Alzheimer's disease is a progressive and fatal brain disease. Since Alzheimer's disease is highly inheritable [10], several GWAS [1,11,22,29] have been conducted to identify the genetic effects, one of which was a family-based study [1] that used affection status and age-at-onset of the disease as the phenotypes of interest in the association analysis. However, given the complex ascertainment condition used in this study [1], i.e. at least two affected pro-bands per family plus additional family-members that are unaffected, the optimal weights cannot be derived here analytically. In such scenarios, simulation studies have to be used to obtain optimal/powerful weight choices.

Based on a set of simulation studies for nuclear family with three offsprings (data not shown), we derived weights for this design that achieve relatively high power level. A weight ratio of 1, i.e. equal weights, seems to provide good statistical power across different genetic models and genetic effect sizes. Our goal was then to examine the sensitivity of p -values of the overall family-test with respect to this weight choice. With the markers of the family-GWA study for AD [1], we assessed the sensitivity of the selected weight ratio over varying weight choices. The sample comprised a total of 1,412 subjects from 439 families. Of these, 1,376 individuals from 410 families were of self-reported European ancestry and the others of African ancestry. In each family, the onset ages of all affected family members are larger than or equal to 50 years, and DNA was available from at least two affected family members.

In Table 8, we confine the weights to those whose w_{FBAT}/w_T are in $[1,2]$, and the most significant N markers are chosen with the proposed method using the given weights. Then the proportion of the common markers is calculated from two sets of the weights. Our results suggest that the SNPs with the smallest p -values do not change much for different weight choices, even for relatively different weight choices.

4 Discussion

While many GWAS have successfully identified novel associations between genes and complex diseases, the replication of genetic-association findings sometimes fails. Insufficient power, population stratification, between-study heterogeneity and aging-varying association [20] can be possible explanations. In particular, population stratification has been an intractable issue in GWAS and their replication studies. It is therefore crucial that the initial analysis of the GWAS that leads to the identification of a new disease gene is robust against such confounding, while maintaining the highest possible power levels. In this communication, we propose a new family-based association test that follows exactly this maxim.

In principle, the proposed method is reminiscent of genomic control for population stratification and the genomic control also can be applied to the screening statistics instead of our rank-based p -value. In genomic control, the variance for the test statistics is assumed to be constantly inflated and its amount is estimated. For our method, it is well known that the p -values follow the uniform distribution under the null hypothesis and we transform the p -values to the rank-based p -values in this context. As a result, we could provide the complete robustness even under the local population stratification while the genomic control fails [35], and it illustrates the superiority of the proposed method in terms of robustness.

However, even though proposed method improves the statistical efficiency under the complete robustness, it is not straightforward to extend it to designs with large pedigrees. For within-family component, we can obtain obviously FBAT. The screening-statistic can be adopted to large pedigrees [18,19] by replacing the off-spring genotypes with their expected genotypes from sufficient statistics for parental genotypes by Rabinowitz and Laird [28]. Then under the conditional mean model [18,19] the usual population-based approach for family design can be applied without breaking the pedigrees to nuclear families.

For affected-only trio design, we proposed to use the public data set for between-family component. However, the population-based approach, such as CA test and QMLS, can handle the public data set as well to improve the statistical power and they may outperform the proposed method in terms of efficiency. However, if there exists heterogeneity in genotyping platform or phenotype diagnosis, the population-based approaches will be violated and we have to be cautious when the population-based approach is adopted. It is necessary to provide the robust p -value for between-family component and the proposed method may be a unique candidate virtually preserving the same efficiency.

While maintaining the robustness of traditional family-based association tests that are not fully efficient, the new overall family-based test achieves power level that is similar to those of population-based studies, or even exceed them when there is population stratification because it prevents over-adjusting population stratification by adjusting population stratification for the statistics only for between-family component. In future work, we will extend the proposed method to multi-marker analysis, haplotype analysis, gene–gene interactions and meta-analysis approaches of multiple GWA studies.

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Fig. 1. Theoretical type-1 rate. *X*-axis and *Y*-axis indicate δ and the logarithm of the ratio w_T / w_{FBAT} with base 10, respectively. For each contour we calculated the maximum of theoretical type-1 error rates which are numerically calculated for GWAS studies with 10,000 SNPs, 45,000 SNPs, and 500,000 SNPs at a genome-wide significance level 0.05. The vertical cross-section panel (to the right of the plot) shows the color legend for the calculated type-1 error rates

Table 1

Numerical optimal weights, w_{FBAT}/w_T is calculated for additive disease mode of inheritance under HWE. h_2 and p_D indicate the heritability and disease allele frequency respectively. Under the additive disease mode of inheritance, we assume that the number of trios is 1,000 and σ^2 is 1

p_D	h^2					
		0.002	0.004	0.006	0.008	0.01
0.05	1.0058	1.0086	1.0108	1.0128	1.0146	
0.10	1.0059	1.0087	1.0110	1.0130	1.0148	
0.15	1.0050	1.0074	1.0094	1.0111	1.0128	
0.20	1.0039	1.0058	1.0074	1.0089	1.0102	
0.25	1.0028	1.0042	1.0055	1.0066	1.0077	
0.30	1.0019	1.0030	1.0039	1.0048	1.0056	
0.35	1.0012	1.0020	1.0028	1.0035	1.0042	
0.40	1.0008	1.0015	1.0020	1.0027	1.0033	
0.45	1.0006	1.0012	1.0017	1.0022	1.0028	
0.50	1.0005	1.0010	1.0015	1.0020	1.0025	

Table 2

Numerical optimal weights. w_{FBAT}/w_T is coagulated for additive (add), recessive (rec) and dominant (dom) disease modes of inheritance. I^2 and p_D indicate the heritability and disease allele frequency respectively. We assume that n is 1,000 and the prevalence is 0.1

f_2/f_0	p_k	$n_{pop} = 500$			$n_{pop} = 1,000$		
		add	rec	dom	add	rec	dom
1.1	0.1	2.180	2.195	2.166	1.691	1.701	1.682
	0.2	2.180	2.190	2.171	1.690	1.697	1.684
	0.3	2.213	2.219	2.207	1.715	1.719	1.711
1.3	0.1	2.148	2.190	2.110	1.670	1.697	1.645
	0.2	2.157	2.184	2.134	1.675	1.694	1.659
	0.3	2.201	2.218	2.185	1.707	1.719	1.696
1.5	0.1	2.118	2.184	2.063	1.650	1.694	1.615
	0.2	2.137	2.179	2.102	1.662	1.691	1.639
	0.3	2.190	2.217	2.168	1.700	1.720	1.684

Table 3

Empirical type-1 error rate ($\times 0.001$). Empirical type-1 error rates are calculated with 10,000 replicates at the genome-wide α -level 0.1 with m SNPs. LIP^p indicates the proposed method using the estimated optimal weights

δ	w_{FRAT}	w_T	m	20	40	60	80	100	500
0	1	2	0.47	0.303	0.237	0.209	0.179	0.179	0.0494
	1	1	2.16	1.21	0.863	0.715	0.586	0.142	
	2	1	3.89	1.97	1.40	1.05	0.878	0.187	
0.5	LIP^p		2.16	1.22	0.868	0.721	0.592	0.143	
	1	2	1.82	1.01	0.682	0.561	0.462	0.118	
	1	1	3.72	1.94	1.28	1.01	0.813	0.174	
	2	1	4.75	2.28	1.55	1.17	0.961	0.196	
	LIP^p		3.8	1.92	1.29	0.102	0.823	0.175	
	0.7	1	2	4.29	2.21	1.42	1.09	0.886	0.189
	1	1	5.34	2.58	1.66	1.26	1.01	0.206	
	2	1	5.42	2.47	1.65	1.23	1.01	0.201	
	LIP^p		5.37	2.58	1.68	1.27	1.03	0.209	
0.9	1	2	14.3	6.69	4.30	3.15	2.45	0.451	
	1	1	9.24	4.22	2.70	1.99	1.57	0.281	
	2	1	6.72	2.94	1.89	1.38	1.12	0.212	
	LIP^p		9.23	4.43	2.73	2.02	1.58	0.282	

Table 4

Empirical type-1 error. The empirical type-1 error rates at 500K GWAS are calculated with 2,000 replicates at the genome-wide α -level 0.05 under both the presence and absence of population stratification. $F_{ST} = 0$ indicates no population stratification

w_{FBAT}/w_T		2:1	1:1	1:2
F_{ST}	0.00	0.0510	0.0505	0.0465
	0.05	0.0455	0.0395	0.0435
	0.10	0.0455	0.0425	0.0405
	0.20	0.0505	0.0450	0.0375
	0.30	0.0370	0.0445	0.0370

Empirical power at 0.01 α -level. The empirical powers at genome-wide α -level 0.01 are calculated for 500K GWAS. LIP^f and LIP^p indicate the proposed method using the estimated optimal weights and equal weights respectively. p_D and h^2 indicate the disease allele frequency and heritability respectively

Table 5

<i>n</i>	h^2	p_D	POP	FBAT	LIP^p	LIP^f
500	0.005	0.1	0.1638	0.0710	0.1588	0.1583
		0.2	0.1608	0.0690	0.1565	0.1558
		0.3	0.1619	0.0710	0.1589	0.1587
	0.01	0.1	0.3705	0.1499	0.3589	0.3587
		0.2	0.3692	0.1548	0.3600	0.3602
		0.3	0.3700	0.1544	0.3620	0.3627
1,000	0.005	0.1	0.3669	0.1559	0.3577	0.3582
		0.2	0.3704	0.1564	0.3620	0.3620
		0.3	0.3727	0.1610	0.3661	0.3662
	0.01	0.1	0.7236	0.3585	0.7122	0.7118
		0.2	0.7263	0.3650	0.7189	0.7181
		0.3	0.7291	0.3683	0.7215	0.7219

Empirical power at 0.01 α -level. The empirical powers at genome-wide α -level 0.01 are calculated for 500K GWAS. We assume that prevalence and disease allele frequency are 0.1 and 0.1 respectively. 1,000 trios with affected offspring are used for FBAT and LIP. For LIP, additional samples from the public data, n_{pop} , are also used. The superscripts 1 and 2 in CA indicate the Cochran–Armitage test with 1,000 cases and 1,000 controls, and 1,500 cases and 1,500 controls, and the subscripts s and u stand for the Cochran–Armitage test with cases/controls and cases/unselected respectively

Table 6

f_2/f_0					$n_{\text{pop}} = 500$		$n_{\text{pop}} = 1,000$	
	FBAT							
	CA_s^1	CA_u^1	CA_s^2	CA_u^2	LIP ^a	LIP ^b	LIP ^a	LIP ^b
1.1	0.0234	0.0190	0.0248	0.0208	0.0204	0.0266	0.0162	0.0298
1.2	0.0624	0.0428	0.0908	0.0732	0.0546	0.0776	0.0572	0.0734
1.3	0.1428	0.1112	0.2346	0.1738	0.1074	0.1466	0.1146	0.1434
1.4	0.2772	0.2002	0.4324	0.3338	0.2204	0.2635	0.2102	0.2719
1.5	0.4320	0.3396	0.6498	0.5264	0.3512	0.4171	0.3578	0.4133

Table 7

Empirical power at 0.01 α -level. The empirical powers at genome-wide α -level 0.01 are calculated for 500K GWAS. We assume that prevalence and disease allele frequency are 0.1 and 0.2 respectively. 1,000 trios with affected offspring are used for FBAT and LIP. For LIP, additional samples from the public data, n_{pop} , are also used. The superscripts 1 and 2 in CA indicate the Cochran–Armitage test with 1,000 cases and 1,000 controls, and 1,500 cases and 1,500 controls, and the subscripts s and u stand for the Cochran–Armitage test with cases/controls and cases/unselected respectively

f_2/f_0					$n_{pop} = 500$		$n_{pop} = 1,000$	
	FBAT							
	CA_s^1	CA_u^1	CA_s^2	CA_u^2	LIP ^a	LIP ^b	LIP ^a	LIP ^b
1.1	0.0404	0.0328	0.0496	0.0386	0.0290	0.0470	0.0312	0.0452
1.2	0.1498	0.1148	0.2276	0.1772	0.1132	0.1526	0.1182	0.1570
1.3	0.3468	0.2664	0.5488	0.4346	0.2764	0.3481	0.2871	0.3551
1.4	0.6000	0.4970	0.8174	0.7092	0.4781	0.5637	0.4953	0.5737
1.5	0.8110	0.7002	0.9488	0.8898	0.6977	0.7670	0.7085	0.7583

Sensitivity analysis in Alzheimer's disease. The mean of the overlapped marker proportion of the most significant c SNPs for each w_{FBAT}/w_T are empirically calculated, where $w_{\text{FBAT}}/w_T \in [1, 2]$ with $\Delta(w_{\text{FBAT}}/w_T)$ fixed

Table 8

Number of top SNPs	$\Delta(w_{\text{FBAT}}/w_T)$				
	0.2	0.4	0.6	0.8	1.0
100	0.89	0.78	0.66	0.58	0.51
500	0.93	0.86	0.80	0.73	0.66
1,000	0.93	0.87	0.80	0.74	0.67
5,000	0.94	0.88	0.83	0.78	0.72
10,000	0.95	0.90	0.85	0.80	0.75