ARTICLE

Two-stage association tests for genome-wide association studies based on family data with arbitrary family structure

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Recently, Steen *et al* proposed a two-stage approach for genome-wide family-based association studies. In the first stage, a screening test is used to select markers, and in the second stage, a family-based association test is performed on a much smaller set of the selected markers. The two-stage method can be much more powerful than the traditional family-based association tests. In this article, we extend the approach so that it can incorporate parental information and can be applied to an arbitrary pedigree structure. We use simulation studies to evaluate the type I error rates and the power of the proposed methods. Our results show that the two-stage approach that incorporates founders' phenotypes has the correct type I error rates, and is much more powerful than the two-stage approach that uses children's phenotypes only. Also, by carefully choosing the number of markers retained in the first stage, the power of a two-stage approach can be much more than that of the corresponding one-stage approach.

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Introduction

Rapid progress in high-throughput genotyping technologies has greatly reduced the cost of genome-wide analyses and is resulting in a boom of large-scale genetic association studies on common disorders. Genome-wide association studies are now widely used approaches in the search for loci that affect complex traits. While genome-wide association studies are promising to mapping complex disease genes,^{1–3} their success will depend on whether the information gain of the increased number of SNPs will be diluted by the multiple-comparison problem.¹ When hundreds of thousands of SNPs are tested for association,

the *P*-values need to be adjusted for controlling type I error rates. Most multiple-testing adjustment approaches, including the Bonferroni correction for controlling family-wise error rate and the method proposed by Benjamini and Hochberg⁴ for controlling false discovery rate (FDR), become more conservative as more tests are performed.

In case-control studies, several authors have proposed a two-stage design that utilizes two samples.^{5,6} The first sample is used to screen and select SNPs for association tests. The association tests are conducted on the selected SNPs by using the second sample, so that the number of association tests is diminished and the correction for multiple testing is less severe. To avoid biased test results, the sample used in the screening step should be independent of the sample used for association testing. For family data, it is possible to create two sources of information using one sample.⁷ Recently, in mapping quantitative trait loci using family data, Steen *et al*¹ proposed an interesting approach that performs the SNP screening and association

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test using the same sample. The basic idea of Steen *et al*'s method is that the screening test based on the traits of the children and between-family genotype scores is statistically independent of the association test that depends on children's trait values and within-family genotype scores. The screening test is used first to select SNPs, and a family-based association test is performed on a much smaller set of the selected SNPs. Since the significance of the association is decided by a transmission/disequilibrium test (TDT) type family-based association test, the results are robust to population admixture.

In this article, motivated by Steen *et al*'s method, we propose two two-stage approaches to analyze genome-wide association data from general pedigrees. The two approaches use two different screening tests in the first stage, but they use the same quantitative pedigree disequilibrium test (QPDT) proposed by Zhang *et al*⁸ as the association test in the second stage. The first screening test, similar to that used by Steen *et al*,¹ does not use parental phenotypes. The second screening test uses phenotypes of the founders in the pedigrees. We use simulation studies to evaluate the performance of the two approaches. Our simulation studies show that by using part of the parental phenotypes, the second approach is consistently more powerful than the first approach, which does not use parental information. Furthermore, by carefully choosing the number of selected SNPs in the first stage, both approaches are more powerful than the one-stage approach that does not use screening tests.

Methods

For the two-stage approaches, if the association test used in the second stage is robust to population admixture, then the two-stage approach will be robust to population admixture regardless of the screening test used in the first stage. Because the number of markers returned in the first step, denoted by L , is fixed in advance and the critical value at the stage is set accordingly, even if the set of L markers tested in the second stage includes a high proportion of false positives due to population stratification, the proportion of those that pass the second stage is not inflated by population stratification and the overall probability that a marker would pass both stages is the nominal test size. In this section, we will propose two two-stage approaches for genome-wide family-based association studies. We will first describe the QPDT, the association test used in the second stage for both the two proposed two-stage approaches.

QPDT: the association test used in the second stage

The TDT⁹ and its extensions for qualitative traits^{10–14} and quantitative traits^{15–19} have become popular tools in testing linkage and association. The main advantage of these TDT type methods is the robustness to population

admixture. For quantitative traits, we chose the QPDT as the association test in our proposed two-stage approaches because it allows arbitrary pedigree structures. The QPDT, proposed by Zhang *et al*,⁸ divides each pedigree into several nuclear families and combines the information from the nuclear families. The QPDT mainly uses the following two types of informative nuclear families in an extended pedigree:

- i Families with both parents available and with at least one parent being heterozygous at the marker being studied.
- ii Families with at most one available parent and with multiple offspring where at least two siblings have different genotypes.

Assume that there are n pedigrees, n_i informative nuclear families in the i th pedigree, and n_{ij} children in the j th informative nuclear family of the i th pedigree. Consider a biallelic marker with two alleles 0 and 1. We use the following notation:

- Y_{ijF} , Y_{ijM} , and Y_{ijk} : the trait value of the father, mother, and the k th child in the j th informative nuclear family of the i th pedigree, respectively.
- X_{ijF} , X_{ijM} , and X_{ijk} : the genotypic score (number of copies of alleles 1) of the father, mother, and the k th child in the j th informative nuclear family of the i th pedigree, respectively.
- $\bar{Y}$ and $\bar{X}$: The average trait value and average genotypic score of all the individuals, respectively.
- $\bar{X}_{ij}$: If parental genotypes are available (the first type of informative nuclear families), $\bar{X}_{ij} = (X_{ijF} + X_{ijM})/2$; otherwise (the second type of informative nuclear families),

$$\bar{X}_{ij} = \frac{1}{n_{ij}} \sum_{k=1}^{n_{ij}} X_{ijk}.$$

By using the notation, the statistic of the QPDT is given by

$$\text{QPDT} = \frac{\sum_{i=1}^n U_i}{\sqrt{\sum_{i=1}^n U_i^2}}, \quad (1)$$

where

$$U_i = \sum_{j=1}^{n_i} U_{ij}$$

and $U_{ij} = \frac{1}{n_{ij}} \sum_{k=1}^{n_{ij}} (Y_{ijk} - \bar{Y})(X_{ijk} - \bar{X}_{ij})$. Under the null hypothesis of no linkage disequilibrium, the QPDT asymptotically follows a standard normal distribution. We can think of random variables U_{ij} as the covariance between the children's trait values and within-family genotypic score, $X_{ijk} - \bar{X}_{ij}$. The QPDT only uses the within-family genotype information, and thus is robust to population admixture.

The screening tests used in the first stage

Although the QPDT only uses informative nuclear families, the screening tests can use all the nuclear families in a pedigree. Let N_i denote the number of nuclear families in the i th pedigree. The two screening test statistics are in the same form as the QPDT statistic. Both the two screening test statistics can be written as

$$T_{\text{SCREEN}} = \frac{\sum_{i=1}^n U_i}{\sqrt{\sum_{i=1}^n U_i^2}},$$

where

$$U_i = \sum_{j=1}^{N_i} U_{ij}.$$

However, U_{ij} are different in the two tests. The definitions of U_{ij} in the two tests are given below:

1. In the first screening test, which uses children's phenotypes only,

$$U_{ij} = \frac{1}{n_{ij}} \sum_{k=1}^{n_{ij}} (Y_{ijk} - \bar{Y})(\bar{X}_{ij} - \bar{X})$$

In this case, the corresponding two-stage approach is called a two-stage test using children's phenotypes only (TTCPO).

2. In the second screening test, which uses children's phenotypes as well as the phenotypes of founders of the pedigrees,

$$U_{ij} = \frac{1}{n_{ij}} \sum_{k=1}^{n_{ij}} (Y_{ijk} - \bar{Y})(\bar{X}_{ij} - \bar{X}) \\ + (Y_{ijF} - \bar{Y})(X_{ijF} - \bar{X})I_{ijF} \\ + (Y_{ijM} - \bar{Y})(X_{ijM} - \bar{X})I_{ijM},$$

where $I_{ijF} = 1$ if the father of the j th family in the i th pedigree is a founder of this pedigree; otherwise, $I_{ijF} = 0$ and I_{ijM} is similarly defined for the mother. In this case, the corresponding two-stage approach is called a two-stage test using founder's phenotypes (TTFP).

Under the null hypothesis of no association, both the screening test statistics follow a standard normal distribution.

Applying the two-stage approaches to genome-wide association studies

Suppose that M SNPs have been genotyped for each sampled individual. In the first stage, we use one of the screening tests to test each of the M SNPs. Select L SNPs with the smallest p -values, where L is a pre-specified number (we will discuss how to choose L later). In the second stage, we apply the QPDT to the L selected SNPs, and declare an SNP as significant at a level of α if the p -value of the QPDT at this marker is less than a threshold $\delta_{L\alpha}$. The threshold $\delta_{L\alpha}$ is determined by controlling the FDR, the ratio of the number of falsely rejected null hypotheses to the total number of rejected null hypo-

theses, at a level of α . To control the FDR at a level of α , we can choose the cutoff $\delta_{L\alpha}$ as follows:⁴

let $p_{(1)}, \dots, p_{(L)}$ be the ordered p -values when we apply the PDT to the L selected markers; then $\delta_{L\alpha} = \max\{p_{(i)}; p_{(i)} \leq i\alpha/L\}$.

Results

We use simulation studies to evaluate the performance of the proposed methods. In the simulations, we consider two family structures. The first one is a nuclear family with one child; the second one is a three generation pedigree as given in Figure 1. This family structure is one of the Centre d'Etude du Polymorphisme Humains (CEPH) pedigrees.²⁰ For each family structure, we use two approaches to generate genotype data. In the first approach, we generate the genotypes of the founders by assuming Hardy-Weinberg equilibrium and linkage equilibrium. Each founder randomly transmits one of his/her two alleles to form the children's genotypes. This means that we generate each allele and each marker independently. The frequency of the minor allele at each marker is a random number between 0.05 and 0.45.

In the second approach, we want to mimic the linkage disequilibrium (LD) structure of real data sets. To generate the genotypes with LD between markers, we use Hudson's ms program,²¹ which assumes the coalescent process with recombination to generate multi-marker haplotypes. We follow Nordborg M, Tavare S,²² and Kimmel G and Shamir R,²³ using a mutation rate of 2.5×10^{-8} per nucleotide per generation, a recombination rate of 10^{-8} per pair of nucleotides per generation, and an effective population size of 10000. Of all the segregating sites, only the ones with a minor allele frequency $> 5\%$ are defined as SNPs and are used in the rest of the analysis. The simulation program produces populations of haplotypes from which genotypes of the founders in each pedigree can be generated. The genotypes for each individual who is not a founder can be formed by her/his parents.

Figure 1 The pedigree structure used in the simulation studies.

Type I error rates

To assess the type I error rates, we generate data under the null hypothesis of no association. Let $Y_1 = (y_F, y_M)$ denote the trait values of the parents and $Y_2 = (y_1, y_2, \dots, y_m)$ denote the trait values of the m children. Assume that (Y_1, Y_2) follows a normal distribution with a mean vector of zero and variance–covariance matrix of

$$\Sigma = \begin{pmatrix} \Sigma_{11} & \Sigma_{12} \\ \Sigma_{21} & \Sigma_{22} \end{pmatrix} \text{ where } \Sigma_{11} = \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix}$$

$$\Sigma_{12} = \Sigma_{21} = \begin{pmatrix} \rho & \dots & \rho \\ \rho & \dots & \rho \end{pmatrix} \text{ and } \Sigma_{22} = \begin{pmatrix} 1 & \dots & \rho \\ \dots & \dots & \dots \\ \rho & \dots & 1 \end{pmatrix}$$

This simulation setting means that the father and mother are independent, and parents with children, and children with children are correlated to the correlation coefficient of ρ . The conditional distribution of $Y_2 = (y_1, \dots, y_m)$ given parental trait values $Y_1 = (y_F, y_M)$ is a normal distribution with mean vector of

$$\mu_c = \Sigma_{21} \Sigma_{11}^{-1} Y_1$$

and variance–covariance matrix of

$$\Sigma_c = \Sigma_{22} - \Sigma_{21} \Sigma_{11}^{-1} \Sigma_{12}.$$

To generate trait values of all individuals in a pedigree, we first generate the trait value of each founder by using a standard normal distribution. The trait values of the other members can be generated by a normal distribution with a mean vector of μ_c and variance–covariance matrix of Σ_c , given the trait values of their parents.

We consider sample sizes of 200, 400, 600, and 800 for nuclear families, and sample sizes of 50, 100, 200, and 300 for large pedigrees. We also consider three different numbers of markers: 1000, 10 000, and 100 000. For each simulation scenario, we generate 1000 samples to estimate type I error rates of the two two-stage approaches by using different values of L (the number of markers retained in the first stage). Note that when $L = M$ (the total number of markers), the two-stage approach is equivalent to the one-stage approach. For 1000 replications, the 95% confidence interval of type I error rate is (0.0362, 0.0638) for the nominal level of 5%. The type I error results based on genotypes generated under Hardy–Weinberg equilibrium are similar to those based on genotypes generated by ms program.²¹ We only report the results based on genotypes generated by ms program. The estimated type I error rates are summarized in Table 1 for nuclear families and Table 2 for large pedigrees. The two tables show that the type I error rates of the TTCPO and TTFP are very consistent with the nominal level of 5% for a small value of L and little more conservative for a larger L . The conservativeness of type I error rates for a large value of L is because of the conservative nature of multiple test adjustments by controlling the FDR. The type I error rates for the TTCPO

Table 1 Type I error rates (in percentage) of the two two-stage tests based on nuclear family samples

Sample size	Number of markers	TTCPO			TTFP		
		L			L		
		1	10	100	1	10	100
200	1000	4.8	5.2	2.8	5.3	4.6	3.3
	10 000	4.6	4.3	2.9	5.4	4.2	2.9
	100 000	3.8	3.0	2.6	4.3	3.8	3.0
400	1000	5.5	5.1	3.5	5.0	4.6	3.7
	10 000	3.9	3.7	3.2	5.3	4.1	3.8
	100 000	6.2	5.7	4.0	5.3	4.5	4.0
600	1000	5.6	4.9	4.4	4.0	3.8	3.9
	10 000	5.3	4.0	3.8	5.3	4.7	3.4
	100 000	4.4	4.2	3.1	5.0	4.5	3.2
800	1000	4.7	4.8	4.6	4.2	5.7	4.6
	10 000	5.7	3.5	3.8	4.9	4.0	5.1
	100 000	4.0	3.9	4.0	5.8	5.5	3.2

This set of simulations uses the genotypes generated by ms software. L denotes the number of markers retained in the first stage.

Table 2 Type I error rates (in percentage) of the two two-stage tests based on general pedigree samples

Sample size	Number of markers	TTCPO			TTFP		
		L			L		
		1	10	100	1	10	100
200	1000	4.8	5.2	2.8	5.3	4.6	3.3
	10 000	4.6	4.3	2.9	5.4	4.2	2.9
	100 000	3.8	3.0	2.6	4.3	3.8	3.0
400	1000	5.5	5.1	3.5	5.0	4.6	3.7
	10 000	3.9	3.7	3.2	5.3	4.1	3.8
	100 000	6.2	5.7	4.0	5.3	4.5	4.0
600	1000	5.6	4.9	4.4	4.0	3.8	3.9
	10 000	5.3	4.0	3.8	5.3	4.7	3.4
	100 000	4.4	4.2	3.1	5.0	4.5	3.2
800	1000	4.7	4.8	4.6	4.2	5.7	4.6
	10 000	5.7	3.5	3.8	4.9	4.0	5.1
	100 000	4.0	3.9	4.0	5.8	5.5	3.2

This set of simulations uses the genotypes generated by ms software. L denotes the number of markers retained in the first stage.

and TTFP also show that the screening tests used in the first stage and the PDT used in the second stage are independent.

Power comparisons

For power comparisons, we first generate genotype data as described in the previous section and then generate trait values according to different disease models as described below. For a pedigree with K members, let $\varepsilon_1, \dots, \varepsilon_K$ be the trait values of the K members generated under the null hypothesis using the method described in the previous section. Assume that the disease locus is a biallelic marker with two alleles D and d . Then, the trait value of the k th member is given by

$$y_k = \beta_1 x_{k1} + \beta_2 x_{k2} + \varepsilon_k,$$

where x_{k1} is the additive genotypic score, that is, $x_{k1} = -1, 0$, or 1 corresponding to genotypes dd, Dd , or DD of the k th

member; x_{k2} is the dominant genotypic score, that is, $x_{k2}=0, 1$, or 0 corresponding to genotypes dd, Dd , or DD of the k th member. We consider three disease models: the additive model in which $\beta_2=0$, the dominant model in which $\beta_1=\beta_2$, and the recessive model in which $\beta_1=-\beta_2$. The values of β_1 and β_2 can be determined by the value of the heritability h and disease models. Consider the additive model as an example. Under the additive model,

$$h = \frac{\text{var}(\beta_1 x_1)}{\text{var}(y)} = \frac{\beta_1^2 \text{var}(x_1)}{\beta_1^2 \text{var}(x_1) + 1} = \frac{2\beta_1^2 p(1-p)}{2\beta_1^2 p(1-p) + 1}$$

and thus

$$\beta_1 = \sqrt{\frac{h}{2(1-h)p(1-p)}},$$

where p denotes the allele frequency of allele D at the disease locus.

In our simulations, we generate genotypes at 100 000 markers for each individual by either one of the two generating methods, and vary the value of L (the number of markers retained in the first step) from 1 to 100 000. The results of $L=100\,000$ are the results of the one-stage approach, the PDT. We consider different sample sizes, different disease models, and different values of heritability h . For each scenario, we use 1000 replicated samples to estimate the power of the tests. To reduce the effect of allele frequency at the disease locus, we randomly choose a marker with minor allele frequency between 0.1 and 0.4 as a disease locus for each replication.

The patterns of power comparisons are similar under the three disease models. We use the additive model as an example to describe the results. The power comparisons of the TTCPO and TTFP are given in Figure 2 for nuclear families and in Figure 3 for large pedigrees. The two figures

Figure 2 Power comparisons of the two-stage tests using 400 and 800 nuclear families with one child in each family. The x-axis denotes the number of markers retained by the screening test in the first stage. The total number of markers is 100 000 (100K). Figures in the left panel, a, c and e, show powers based on genotype data generated under the assumption of linkage equilibrium between markers. Figures in the right panel, b, d and f, show powers based on genotype data generated by using ms software. h denotes the heritability.

show that the TTFP, which uses the founders' phenotypes consistently outperforms the TTCPO for all the scenarios we considered. In most cases, the power of the TTFP is substantially more than that of the TTCPO. For example, the power of the TTFP is 70% while the power of the TTCPO is 10% when the sample contains 800 nuclear families and the heritability $h=0.03$, the number of markers retained in the first stage $L=1$, and the genotypes are generated under the assumptions of the Hardy–Weinberg equilibrium and the linkage equilibrium. Comparing the two two-stage approaches with the one-stage approach, that is the QPDT, by carefully choosing L , both of the two-stage approaches are more powerful than the one-stage approach in all the cases. However, the power of the two-stage approaches depends on L , the number of markers retained in the first stage, and the optimal value of L depends on the LD structure of the genotypes. Steen *et al*¹

suggested using $L=10$. Our results for the genotypes generated under linkage equilibrium suggest a similar value of L . A value between 10 and 20 is a good choice for L . However, in the more realistic situation where there is LD between markers, our results suggest a much larger L . It seems that the best choice for L is between 100 and 500. The reason may be that, in this case, several markers may have a strong LD with the disease locus and thus have small p-values. If we only retain a few markers in the first stage, we may pick up one or more markers that have strong LD with the disease locus but not the disease locus itself.

Discussion

The main purpose of this article is to extend the two-stage method proposed by Steen *et al*¹ so that the method can

Figure 3 Power comparisons of the two-stage tests using 100 and 200 pedigrees. The x-axis denotes the number of markers retained by the screening test in the first stage. The total number of markers is 100 000 (100K). Figures in the left panel, a, c and e, show powers based on genotype data generated under the assumption of linkage equilibrium between markers. Figures in the right panel, b, d and f, show powers based on genotype data generated by using ms software. h denotes the heritability.

incorporate parental information and can be easily applied to large pedigree data. For this purpose, we proposed two two-stage approaches, the TTCPO and TTFP. The basic assumption to guarantee the correct type I error rates of a two-stage approach is that the test used in the first stage is statistically independent of the test used in the second stage. The TTCPO, which uses children's phenotypes only, and the TTFP, which uses phenotypes of children as well as the founders in the pedigrees, have correct type I error rates in all the cases we considered. We further compare the power of the two valid approaches, the PPCPO and TTFP. Our results show that by incorporating the founders' phenotypes, the TTFP is consistently more powerful than the PPCPO. In some cases, the power of the TTFP is several times more than that of the PPCPO.

Although most of genome-wide association studies use a case-control design, genome-wide family-based association studies are still necessary.²⁴ Family-based genome-wide association studies usually use nuclear families extracted from pedigrees of previous linkage studies. The phenotype data of the nuclear families from previous linkage studies often include parental phenotypes. In this case, our proposed TTFP is feasible and more powerful than the method proposed by Steen *et al.*¹ Although general pedigrees have not been seen in genome-wide association studies, they may be used in the follow-up studies, and thus our method is necessary.

One remaining question is choosing the value of L , the maximum number of markers retained in the first stage. Our simulation results show that there is no unique answer in choosing an optimal L . If there is weak LD between adjacent markers, the values between 10 and 20 may be a good choice for L . If there is strong LD between adjacent markers, the optimal value of L should be much larger, 100–500 as suggested by our simulation study. In conclusion, we need further investigations on choosing the optimal value of L .

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