ARTICLE TYPE

A Simple Yet Efficient Parametric Method of Local False Discovery Rate Estimation Designed for Genome-Wide Association Data Analysis

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Summary

In genome-wide association studies (GWAS), hundreds of thousands of genetic features (genes, proteins, etc.) in a given case-control population are tested in favor of the null hypothesis that there is no association between each genetic marker and a specific disease. A popular approach in this regard is to estimate local false discovery rate (LFDR), the posterior probability that the null hypothesis is true, given an observed test statistic. Assuming a certain structure for the underlying model, covering many situations in genome-wide association studies, we use the method of moments and introduce a simple, fast and efficient method for LFDR estimation. We evaluate the performance of the proposed approach by performing two different simulation strategies. As well, we examine the practical utility of the proposed algorithm by analyzing a comprehensive 1000 genomes-based genome-wide association data containing approximately 9.4 million single nucleotide polymorphisms, and a microarray data set consisting of genetic expression levels for 6033 genes for prostate cancer patients.

KEYWORDS:
disease association, empirical Bayes, local false discovery rate, method of moments, multiple hypothesis testing

1 | INTRODUCTION

Genetic association studies deal with investigating an association between some disease traits and some genetic features, including genes, proteins, lipids and single nucleotide polymorphisms (SNPs). The investigation follows certain strategies to determine whether there exists some kind of statistical association. In case-control studies, the investigation is started by the determination of differences between the frequency of alleles or genotypes at genetic marker loci in individuals from a given population. Significant differences then reflect strong statistical evidence to claim for existence of association. In this paper, we focus on the analysis of genetic SNP data from population-based genome-wide association studies (GWAS). However, one may apply the results to other data sets, as long as the underlying model follows the structure considered in this paper.

In GWAS, \( N \) SNPs (with \( N \) being usually hundreds of thousands) are genotyped in a given case-control population, and are tested in favor of the null hypothesis \( H_{0i}, i = 1, \ldots, N \), that there is no association between SNP \( i \) and the disease, and against the alternative hypothesis \( H_{1i} \), that there is such an association in that population. For an individual SNP \( i \), the classic statistics deals with testing \( H_{0i} \) versus \( H_{1i} \), by verifying whether a test statistic \( x_i \) falls inside some critical region \( C_{a} \), where \( a \) is the significance
level (type-I error or false positive rate). As an example, if \( x_i \) represents an estimated allelic odds ratio (OR) for SNP \( i \), then a critical region may be presented by \( C_a = \{ x_i : x_i < \chi_1^{2,1-a/2} \text{ or } x_i > \chi_1^{2,1-a/2} \} \), where \( \chi_1^{2,1-\gamma} \) denotes \( 100(1-\gamma)\% \)-quantile of the chi-square distribution with one degree of freedom. Alternatively, the single hypothesis problem might be tested by comparing the significance level \( \alpha \) with the resulting p-value \( p_i \), the smallest value of \( \alpha \) such that \( x_i \in C_a \). According to Fisher’s scale of evidence for interpreting p-values, the less p-value, the more evidence against the null hypothesis \( H_{0i} \). Either way, the procedure is simple and convenient to use, but the approach leads to a high-rate of false discoveries. To overcome this challenging situation, several improvements on a set of given p-values have been introduced in the literature, see \( \ref{9}, \ref{41}, \ref{42} \) and \( \ref{43} \). Alternative to p-value adjustment procedures, is the false discovery rate (FDR) estimation introduced by Benjamini and Hochberg \( \ref{15} \), aiming at controlling the expected proportion of falsely rejected hypotheses. This seminal work led to many developments. For example, Storey \( \ref{16} \) develops a Bayesian approach for estimating FDR and Efron et. al \( \ref{17} \) outline an empirical Bayesian interpretation. The latter defines FDR as the posterior probability that a null hypothesis \( H_{0i} \) is true given that an observed test statistic \( x_i \) falls within some critical region \( C \), i.e., \( P(H_{0i} | x_i) = \text{true} | C \). In case the critical region \( C \) consists of only one point, FDR is referred to by the term Local FDR (LFDR), see Efron \( \ref{16} \) and Padilla and Bickel \( \ref{44} \).

In the problem of testing \( N \) SNPs against the null hypothesis \( H_{0i}, i = 1, \ldots, N \), we assume that each of the \( N \) SNPs is unassociated (with some disease) with prior probability \( \pi_0 \). We further suppose that the test statistic \( x_i \) follows a null density, say \( f_0 \), with chance \( \pi_0 \) and a non-null density, say \( f_1 \), with chance \( \pi_1 = 1 - \pi_0 \). Then, LFDR for each SNP \( i \) is defined as follows

\[
\psi_i = P(H_{0i} \text{ is true} | x_i) = \frac{\pi_0 f_0(x_i)}{f(x_i)},
\]

where \( f(x_i) = \pi_0 f_0(x_i) + \pi_1 f_1(x_i) \). In general \( f_0 \) is assumed to be known (e.g., standard normal density, central chi-square density with some known degree(s) of freedom, etc.), \( f_1 \) is assumed to be a known density function with some unknown parameter(s) (e.g., normal density with unknown mean and/or unknown variance, chi-square density with some known/unknown degree(s) of freedom with some unknown non-centrality parameter, etc.), and \( \pi_0 \) is an unknown parameter. Such unknown parameters need to be estimated before making any inferences. Estimated values are then replaced in equation (1) and the resulting estimated LFDR, say \( \hat{\psi}_i \), is compared to some pre-determined threshold. SNPs not passing the pre-specified threshold are deemed to be associated with the underlying disease.

Different strategies have been used for LFDR estimation in the literature. Pan et al. \( \ref{45}, \ref{46} \) and Efron \( \ref{16}, \ref{17} \) perform the estimation task using a discrete mixture model, and Muralidharan \( \ref{47} \), Padilla and Bickel \( \ref{44} \) and Yang et al. \( \ref{48} \) use the maximum likelihood (ML) approach. Bicke \( \ref{44} \) summarizes strengths and weakness of classic and empirical Bayes estimation approaches.

In this paper, we assume a certain structure for the underlying model. We assume a multiple hypothesis testing problem in which \( N \) SNPs in a given case-control population are tested in favor of the null hypothesis \( H_{0i} \) against a certain disease. We assume that \( \pi_0 \), the true proportion of unassociated SNPs, is unknown. Further, we assume that \( f_0 \) represents a central chi-square density function with some known degree(s) of freedom \( \lambda \) and \( f_1 \) represents a non-central chi-square density function with \( \nu \) degree(s) of freedom and an unknown non-centrality parameter \( \lambda \). We will discuss in the forthcoming section that these assumptions are not restrictive and are made in many genetic association studies.

Being concentrated on the chi-square model, Padilla and Bickel \( \ref{44} \) as well as Karimnezhad and Bicke \( \ref{44} \) assume that the test statistics \( x_i, i = 1, \ldots, N \), are independent and estimate the corresponding LFDR by using the ML approach, i.e.,

\[
\hat{\psi}_i = \frac{\hat{\pi}_0 f_0(x_i)}{\hat{\pi}_0 f_0(x_i) + (1 - \hat{\pi}_0) f_1(x_i)},
\]

where

\[
(\hat{\pi}_0, \hat{\lambda}) = \arg \max_{\pi_0 \in [0,1], \lambda \in [c,d]} \prod_{i=1}^{N} \left( \pi_0 f_0(x_i) + (1 - \pi_0) f_1(x_i) \right),
\]

in which \( c \) and \( d \) are known bounds. Although this approach leads to somehow sensible estimators, the independency assumption might be unrealistic in genetic association studies due to linkage disequilibrium (LD). Also, the resulting LFDR estimates highly depend on the bounds of \( c \) and \( d \), and inappropriate choices for these bounds can negatively affect the estimation precision. As another downside, this estimation procedure is time-consuming, and the processing time increases with the number of SNPs as well as the length on the interval \([c, d]\). Alternative to this approach, we provide a simple yet efficient algorithm that estimates LFDRs without assuming independency. We theoretically show that the resulting estimator has a high precision as long as \( N \), the number of SNPs to be tested, is large. Not only is the proposed approach fast, but also it provides an explicit form of estimators of the proportion rate \( \pi_0 \) and the non-centrality parameter \( \lambda \). As a result, unlike many algorithms including the ML approach...
of Padilla and Bickel and the histogram-based (HB) approach of Efron, it provides an explicit form of the corresponding LFDR estimator.

The structure of this paper is as follows. In Section 2, we quickly review frequent measures for genetic association studies used in the literature. We discuss that many genetic association studies reduce to a multiple hypothesis testing problem in a chi-square model. In Section 3, we present our proposed empirical Bayes approach. Section 4 is devoted to evaluating performance of the proposed approach. We follow two different simulation strategies and use the mean squared error (MSE) as a common measure of performance. In Section 5, we apply the proposed approach and analyze two different sets of real data, including a set of microarray data and another set of coronary artery disease data. We wrap up the paper by providing some discussion and concluding remarks in Section 6.

2 COMMON MEASURES FOR ASSOCIATION

Consider a diallelic marker locus with a typical allele (wild-type) A and the alternative (risk) allele B, and let \( p \) represent the frequency of the risk allele, i.e., \( p = P(B) \) and \( 1 - p = P(A) \). Denote the corresponding genotypes by \( G_0 = AA, G_1 = AB \) and \( G_2 = BB \) (we do not distinguish between \( AB \) and \( BA \)). Then, genotype frequencies in the population are given by \( g_j = P(G_j) \), \( j = 0, 1, 2 \), where \( g_0 = (1 - p)^2 + p(1 - p)F \), \( g_1 = 2p(1 - p)(1 - F) \) and \( g_2 = p^2 + p(1 - p)F \), in which \( F \) is Wright’s coefficient of inbreeding. For humans, \( F \) is usually taken to be between 0 and 0.05. Under Hardy-Weinberg (HW) equilibrium, \( F = 0 \). Thus, when HW proportions hold in the population, \( g_0 = (1 - p)^2, g_1 = 2p(1 - p) \) and \( g_2 = p^2 \). Let the prevalence of the disease be \( k = P(\text{case}) \), and define \( v_i = P(\text{case}|G_j) \), the probability of having a disease given a specific genotype at the marker for genotype \( G_j \). i.e., \( 0, 1, 2 \). Obviously, \( k = \sum_{i=0}^{2} v_i g_i \). Depending on a chosen genetic model, the penetrances \( v_i \) have certain relationships with themselves. If the genetic model is additive, then \( v_1 = \frac{v_0 + v_2}{2} \). For recessive, multiplicative and dominant models, \( v_1 = v_0, v_1 = \sqrt{v_0 v_2} \) and \( v_1 = v_2 \), respectively, see Zheng et al.20

Genetic association is usually measured for each individual SNP separately. The data for each SNP can be summarized in a contingency table of either genotype counts or allele counts by disease status (case or control). Table 1 represents genotype counts at marker \( M \) based on a sample of \( r \) cases and \( s \) controls. Intuitively, the chance of observing genotype \( G_i \) provided that an individual is known to belong to the case group is estimated by \( \frac{r_i}{r} \), which is in fact the ML estimate of \( p_j = P(G_j|\text{case}) \). Similarly, \( q_j = P(G_j|\text{control}) \) is the true chance of having genotype \( G_i \) for a control individual which, with the notations in Table 1, is estimated by \( \frac{s_i}{s} \). It is easy to verify using the Bayes principle that \( p_j = \frac{r_i g_i}{r} \) and \( q_j = \frac{(1-i)g_i}{1-s} \). Table 2 represents allele counts at marker \( M \) based on a sample of \( r \) cases and \( s \) controls.

Contingency tables play a key role in summarizing genetic association data. Under the null hypothesis that there is no association between genotypes in Table 1 (or alleles in Table 2) and the disease, the same relative genotype (or allele) frequencies in both case and control groups are expected. Contingency tables also allow to summarize data using different models of penetrance. Perhaps Pearson’s test is the most convenient association test in contingency tables. Pearson’s test statistic for the genotypes in Table 1 can be presented by

\[
T_p = \sum_{j=0}^{2} \frac{(r_j - n_j r/n)^2}{n_j r/n} + \sum_{j=0}^{2} \frac{(s_j - n_j s/n)^2}{n_j s/n},
\]

which approximately follows a chi-square distribution with two degrees of freedom. In the same way, the test can be applied to allele counts in Table 2, and the resulting test statistic approximately follows a chi-square distribution with one degree of freedom, see Zheng et al.20 and Clarke et al.21 among many others. In fact, any test statistic in genetic association studies by means of a contingency table follows a chi-square distribution with at most two degrees of freedom, see Table 2 of Clarke et al.21. Readers may also refer to Clarke et al.21 for a summary of strengths and weaknesses of allelic and genotypic tests of association, as well as the differences between the different models of penetrance.

As an alternative association test, Cochran-Armitage trend (CAT) test is used in situations where some kind of trend in risk of developing the disease with increasing number of the risk allele in three genotypes is determined. The CAT test assigns some scores \( w_1, w_2 \) and \( w_3 \) to the three genotypes \( G_0, G_1 \) and \( G_2 \) with the condition that \( w_0 \leq w_1 \leq w_2 \) and \( w_0 < w_2 \), and looks for weighted differences between the genotype frequencies in cases and in the union of case and control samples. The corresponding test statistic is given by

\[
T_C = \frac{r}{n} \left[ \frac{\sum_{j=0}^{2} w_j (r_j s - s_j r)}{\sum_{j=0}^{2} w_j n_j (n - n_j) - 2 \sum_{j=1}^{2} \sum_{i=j+1}^{3} w_i w_j n_i n_j} \right]^2.
\]
which under the null hypothesis of no association follows a chi-square distribution with one degree of freedom. The weights \( w_0, w_1 \) and \( w_2 \) are chosen to test a particular type of association. For example, to test whether the allele \( A \) is recessive, the choices would be \( w_0 = 0, w_1 = 0 \) and \( w_2 = 1 \). See Clarke et al.\(^{13}\) and Zheng et al.\(^{20}\) for more details.

**TABLE 1** A typical 2×3 table with \( R \) cases and \( N \) controls.

|        | AA   | AB   | BB   | Total |
|--------|------|------|------|-------|
| Case   | \( s_{0i} \) | \( s_{1i} \) | \( s_{2i} \) | \( s \) |
| Control| \( n_{0i} \) | \( n_{1i} \) | \( n_{2i} \) | \( n \) |
| Total  | \( n_0 \) | \( n_1 \) | \( n_2 \) | \( n \) |

**TABLE 2** Typical allele counts of case-control samples for a single marker

|        | A     | B     | Total |
|--------|-------|-------|-------|
| Case   | \( 2r_0 + r_1 \) | \( 2r_2 + r_1 \) | \( r \) |
| Control| \( 2s_0 + s_1 \) | \( 2s_2 + s_1 \) | \( s \) |
| Total  | \( 2n_0 + n_1 \) | \( 2n_2 + n_1 \) | \( n \) |

Odds ratios (ORs) are another common measure of association between genotypes and diseases. ORs compare the odds of disease in an individual carrying one genotype to the odds of disease in an individual carrying a different genotype. Thus, for a diallelic marker, the following two genotypic ORs can be defined

\[
OR_i = \frac{v_i(1-v_0)}{v_0(1-v_i)}, \quad i = 1, 2. \tag{4}
\]

\( OR_1 \) compares the odds of disease between individuals carrying genotype \( AB \) and those carrying \( AA \), and \( OR_2 \) compares the odds of disease between individuals carrying genotype \( BB \) and those carrying \( AA \). In case of no association, \( OR_1 = OR_2 = 1 \).

According to the data in Table 1 ORs can be estimated by \( \overline{OR}_i \) with \( \text{Var}(\overline{OR}_i) = \frac{1}{r_i} + \frac{1}{s_i} + \frac{1}{n_i}, \quad i = 1, 2 \). Then,

\[
T_{OR_i} = \frac{\log \overline{OR}_i - \log OR_i}{\sqrt{\text{Var}(\log \overline{OR}_i)}} \sim N(0, 1), \tag{5}
\]

or equivalently \( T^2_{OR} \sim \chi^2_1 \). An OR can also be defined to the allele counts in Table 2. If so, an estimated OR is given by \( \overline{OR} \) with

\[
\text{Var}(\overline{OR}) = \frac{1}{2r_0 + r_1} + \frac{1}{2r_2 + r_1} + \frac{1}{2s_0 + s_1} + \frac{1}{2s_2 + s_1}. \tag{6}
\]

Similarly, the corresponding test statistic follows a chi-square distribution with one degree of freedom.

A more flexible analysis for GWAS is based on the logistic regression model. Let the binary random variable \( Y_i \) represent whether \( i \)th individual belongs to the case group \( (Y_i = 1) \) or to the control group \( (Y_i = 0) \), and let \( X_i \) denote the genotype of individual \( i \) for an arbitrary SNP so that \( X_i(G_0) = 0, X_i(G_1) = 1 \) and \( X_i(G_2) = 2 \). Then, the logistic model is defined as \( \ln \frac{\theta_i}{1 - \theta_i} = \beta_0 + \beta_1 X_i \), where \( \theta_i = E[Y_i|X_i] \) is the expected value of phenotype given a genotype for an arbitrary SNP. With this setting, the multiple hypothesis testing problem reduces to testing \( H_{0i} : \beta_1 = 0 \) vs \( H_{1i} : \beta_1 \neq 0, \quad i = 1, \ldots, N \). The corresponding test statistic is computed by \( T_L = \frac{\beta_1}{\sqrt{\text{Var}(\hat{\beta}_i)}} \). Under the null hypothesis of no association, it follows that \( T_L \sim N(0, 1) \) or equivalently \( T^2_L \sim \chi^2_1 \). For details see Padilla and Bickel\(^{10}\), Yang et al.\(^{15}\) and Karimnezhad and Bickel\(^{12}\).

### 3 A NOVEL EMPIRICAL BAYESIAN METHOD

As discussed in the preceding section, many genetic association studies reduce to a multiple hypothesis testing problem in which the corresponding test statistics follow a chi-square distribution. To conduct such a hypothesis testing problem, we propose to apply and estimate LFDRs by using a simple and efficient empirical Bayes approach, as we present below.
Suppose that $N$ SNPs have been genotyped in a case-control population, and that the goal is to test the null hypothesis $H_0$, $i = 1, \ldots, N$, indicating that there is no association between SNP $i$ and the disease, against its alternative hypothesis $H_1$. Suppose that for each SNP $i$, the test statistic $x_i$ has already been computed using any of the approaches reviewed in the preceding section. Then, let an indicator variable $\mu_i$ represent whether $i$th null hypothesis is true in nature, i.e., $H_0 : \mu_i = 0$ and $H_1 : \mu_i = 1$. Further, let $\pi_0 \in [0, 1]$ be the true proportion of SNPs not associated with the disease, and define

$$\mu_i = \begin{cases} 0 & \text{with probability } \pi_0, \\ 1 & \text{with probability } 1 - \pi_0. \end{cases}$$

In fact, this indicator variable assigns some chance $\pi_0$ to each null hypothesis to be true. Define $\theta$ to be a two-state variable so that it takes 0 if $\mu_i = 0$, and takes a positive value $\lambda$ if $\mu_i = 1$. Further, assume that the test statistic $X_i$ follows $\chi^2_{\nu, \theta}$, the chi-square distribution with $\nu$ degree(s) of freedom and non-centrality parameter $\theta$. This model can be expressed by the following hierarchical model

$$\begin{align*}
X_i | \theta & \sim \chi^2_{\nu, \theta}, \quad i = 1, \ldots, N, \\
\theta | \mu_i & \sim \mu_i \delta_{\mu_i} + \lambda \delta_{1-\mu_i}, \\
\mu_i & \sim \text{Bernoulli}(1 - \pi_0),
\end{align*}$$

where

$$\delta_a = \begin{cases} 0 & \text{if } a \neq 0, \\ 1 & \text{if } a = 0. \end{cases}$$

Such a hierarchical Bayes model has already been applied in detecting variants in the analysis of next generation sequencing data.

As we reviewed earlier, the degree(s) of freedom $\nu$ in many genetic association studies reduces to one. Concentration on the chi-square distribution with one degree of freedom leads to the following nice simplification in LFDR estimation.

**Theorem 1.** Let $X_i, i = 1, \ldots, N$, follow the chi-square distribution with one degree of freedom and the non-centrality parameter $\lambda$.

(i) The density function of an observation $x_i$ can be expressed by

$$f_x(x_i) = \frac{1}{2 \sqrt{2\pi x_i}} \left( e^{-\frac{1}{2}(\sqrt{x_i} - \lambda)^2} + e^{-\frac{1}{2}(\sqrt{x_i} + \lambda)^2} \right).$$

(ii) Let $x_i$ be an observation from the mixture density $f(x_i) = \pi_0 f_0(x_i) + (1 - \pi_0) f_x(x_i)$. Then, the LFDR based on observing $x_i$ is given by

$$\psi(x_i) = \pi_0 \left( \pi_0 + (1 - \pi_0) e^{-\frac{1}{2} \cosh(\sqrt{2x_i})} \right)^{-1},$$

and for a given threshold $u$, $\psi(x_i) < u$ if and only if $x_i > h_u(\pi_0, \lambda)$, where

$$h_u(\pi_0, \lambda) = \frac{1}{\lambda} \left[ \ln \left( k_u(\pi_0, \lambda) + \sqrt{k_u^2(\pi_0, \lambda) - 1} \right) \right]^2,$$

with $k_u(\pi_0, \lambda) = \pi_0 e^\frac{u}{2} \left( \frac{e^{u} - 1}{1 - \pi_0} \right)$.

**Proof.** The proof is straightforward and hence omitted.

Equation (7) is in fact a simplified version of equation (1) applicable to genetic association studies. In order to be able to estimate the LFDR, the parameters $\pi_0$ and $\lambda$ in (7) need to be estimated. In this regard, we propose estimating the LFDR using the method of moment (MM) estimation, which suggests that unknown parameters in a model should be estimated by matching theoretical moments with the appropriate sample moments.

**Theorem 2.** With the setting of Theorem 1 let $m_1 = \frac{1}{N} \sum_i X_i$ and $m_2 = \frac{1}{N} \sum_i X_i^2$ represent the first and the second moments, respectively. Then, MM estimators of $\lambda$ and $\pi_0$ are given by

$$\hat{\lambda} = \frac{m_2 - 3}{m_1 - 1} - 6, \quad \hat{\pi}_0 = 1 - \frac{m_1 - 1}{\hat{\lambda}}.$$
By using the properties of conditional expectation, observe that
\[ E[X_i] = E_\theta[E[X_i|\theta]] = 1 + E_{\mu_i}[E[\theta|\mu_i]] = 1 + (1-\pi_0)E_\theta[\theta|\mu_i = 1]. \]
and
\[ E[X_i^2] = E_\theta[E[X_i^2|\theta]] = E_{\mu_i}[E_\theta((\theta + 1)^2 + 2(1+2\theta)|\mu_i]] = 6m_1 - 3 + (1-\pi_0)E_\theta[\theta^2|\mu_i = 1]. \]

Now, using that fact that \( E_\theta[\theta|\mu_i = 1] = \lambda \) and \( E_\theta[\theta^2|\mu_i = 1] = \lambda \), along with equating the above expectations with the first and second moments, the above equations lead to (9).

Consistency of the MM estimators guarantees that \( \hat{\lambda} \) and \( \hat{\pi}_0 \) converge in probability to \( \lambda \) and \( \pi_0 \), respectively. We show in the next section that \( \hat{\lambda} \) and \( \hat{\pi}_0 \) estimate the true parameters \( \lambda \) and \( \pi_0 \) very well.

To make an inference regarding association between \( i \)-th SNP and the disease, one may compute the estimated LFDR \( \hat{\gamma}_i \) by replacing estimates of \( \lambda \) and \( \pi_0 \) from equation (9) into equation (7). Therefore, if for a given threshold \( u \), \( \hat{\gamma}_i < u \), the null hypothesis \( H_0 \) is rejected. Otherwise, there is no evidence of association. An alternative approach would be to replace estimates of \( \lambda \) and \( \pi_0 \) from equation (9) into \( h_u(\pi_0, \lambda) \) in equation (8). Then, \( H_0 \) is rejected only if the test statistic \( x_i \) is less than \( h_u(\hat{\pi}_0, \hat{\lambda}) \). The second approach is simpler and more convenient, and unlike the existing methods in the literature, it allows for performing multiple hypothesis testing by just comparing each of the test statistics \( x_i \) with a purely data-based threshold, i.e., \( h_u(\hat{\pi}_0, \hat{\lambda}) \).

The threshold \( u \) in (8) can be chosen according to a subjective belief. A conventional choice would be to choose \( u = 0.2 \) to identifying “interesting cases”, see Efron. It also can be chosen based on an objective belief. In this regard, Karimnezhad and Bickel follow a decision theoretic approach in which for a binary decision rule \( \delta_i \), the null hypothesis \( H_0 \) is rejected if \( \delta_i = 1 \), and is not rejected if \( \delta_i = 0 \). They used the following loss function
\[
L(\mu_i, \delta_i) = \begin{cases} 
0 & \text{if } \delta_i = \mu_i = 1 \text{ or } \delta_i = \mu_i = 0, \\
{l_f} & \text{if } \delta_i = 1, \mu_i = 0, \\
{l_{II}} & \text{if } \delta_i = 0, \mu_i = 1,
\end{cases}
\]
where \( l_f \) and \( l_{II} \) are loss values incurred due to making type I and type II errors, respectively. The resulting Bayes estimator of the parameter \( \mu_i \) is then given by
\[
\delta_i^u = \begin{cases} 
1 & \text{if } \hat{\gamma}_i \leq \frac{l_f}{l_f + l_{II}}, \\
0 & \text{if } \hat{\gamma}_i > \frac{l_f}{l_f + l_{II}}.
\end{cases}
\]
(10)

Now, it can be verified using Theorem that the Bayes rule \( \delta_i \) in equation (10) reduces to the following Bayes rule
\[
\delta_i = \begin{cases} 
1 & \text{if } x_i \geq h_u(\hat{\pi}_0, \hat{\lambda}), \\
0 & \text{if } x_i < h_u(\hat{\pi}_0, \hat{\lambda}),
\end{cases}
\]
(11)
with \( u = \frac{l_f}{l_f + l_{II}} \). This Bayes rule is simpler and more convenient than the Bayes rule \( \delta_i \) in equation (10), due to the fact that it is based on the observed test statistic \( x_i \) and estimates of \( \pi_0 \) and \( \lambda \), which are available through the equation (9). In fact, equation (11) illustrates that, unlike many existing algorithms in the literature, one may perform a multiple hypothesis testing comparison by just comparing their observed test statistics \( x_i \) and the data-based function \( h_u(\hat{\pi}_0, \hat{\lambda}) \).

4 | SIMULATION

To illustrate the performance of the proposed LFDR estimation approach, we conduct simulations using two different strategies.
4.1 First simulation study

We follow the simulation strategy used in Karimnezhad and Bickel\[17\]. We take advantage of the fact that squared of log transformation of OR follows a chi-square distribution with one degree of freedom (see equation (5)), and that as reviewed in Section 2, many algorithms in genetic association studies reduce to a chi-square model with one degree of freedom. For each iteration in our simulation study, we assume there are a total number of \( N \) SNPs to be tested, of which \( N_0 \) SNPs are unassociated. We generate \( z_i \) from \( N(0, \sigma^2) \)-distribution, where \( \sigma^2 \) is known, and for \( i = 1, \ldots, N_0 \), \( OR = 1 \), and for \( i = N_0 + 1, \ldots, N \), \( OR \neq 1 \). Obviously, \( x_i = \left( \frac{z_i}{\sigma} \right)^2 \sim \chi^2_{1, \lambda_i} \), where for \( i = 1, \ldots, N_0 \), \( \lambda = 0 \), and for \( i = N_0 + 1, \ldots, N \), \( \lambda = \log(OR) \). We take the steps in Algorithm 1.

We carried out different simulations with different parameters. Figures 1a-1b reflect the simulation results based on taking \( N_0 = 250, 500, 1000(1000)9000, 9500, 9750, N = 10000, n = 100 \) and \( OR = 1.25, 1.5 \). Because from (6), variance of OR is expected to be a very small number, we took \( \sigma^2 = 0.01, 0.02 \). By these choices of OR and \( \sigma^2 \), the true non-centrality parameter \( \log(OR) \) takes one of 2.49, 4.98, 16.44, 8.22 values. From Figure 1a, we observe that for different values of \( \pi_0 \) (especially when \( \pi_0 \geq 0.80 \)), \( \hat{\pi}_0 \) estimates the true \( \pi_0 \) very well. Remarkably, as the true \( \pi_0 \) increases, MSE of the corresponding MM estimator decreases. As well, it is evident from Figure 1b that for different values of \( \pi_0 \), \( \hat{\lambda} \) estimates the true non-centrality parameter \( \lambda \) quite well. Although for high values of \( \pi_0 \), \( \hat{\lambda} \) yields to an increase in \( MSE_{\pi_0} \), \( \hat{\pi}_0 \) leads to a decrease in \( MSE_{\pi_0} \). However, the MM estimated values of \( \pi_0 \) and \( \lambda \) lead to very low \( MSE_{\pi_0} \), values, as presented in Figure 2. Also, as observed from this figure, a decrease in \( \sigma^2 \) or an increase in OR leads to a decreased MSE.

Algorithm 1 First simulation strategy.

Step 1. Specify \( N \), \( N_0 \), \( OR \) and \( \sigma^2 \).

Step 2. Take \( j = 1 \).

Step 3. Generate \( z_1, \ldots, z_{N_0} \) from \( N(0, \sigma^2) \)-distribution.

Step 4. Generate \( z_{N_0+1}, \ldots, z_N \) from \( N(0, \sigma^2) \)-distribution.

Step 5. Compute \( x_i = \left( \frac{z_i}{\sigma} \right)^2 \), \( i = 1, \ldots, N \), the chi-square test statistics.

Step 6. Compute \( \hat{\pi}_0 \) and \( \hat{\lambda} \) using equation (9).

Step 7. Compute \( \hat{\psi}_1, \ldots, \hat{\psi}_N \) by replacing \( \hat{\pi}_0 \) and \( \hat{\lambda} \) into equation (7).

Step 8. Compute errors in estimating \( \pi_0 \), \( \lambda \) and \( \psi_i \) by \( E^j_{\pi_0} = (\hat{\pi}_0 - \pi_0)^2 \), \( E^j_{\lambda} = (\hat{\lambda} - \lambda)^2 \) and \( E^j_\psi = \frac{1}{N} \sum_{i=1}^{N} (\hat{\psi}_i - \psi_i)^2 \), respectively.

Step 9. Increase \( j \) by one and repeat Steps 3 to 8 for \( n \) times. Then, compute

\[
MSE_{\pi_0} = \frac{1}{n} \sum_{j=1}^{n} E^j_{\pi_0}, \quad MSE_{\lambda} = \frac{1}{n} \sum_{j=1}^{n} E^j_{\lambda}, \quad MSE_\psi = \frac{1}{n} \sum_{j=1}^{n} E^j_\psi.
\]
Second simulation study

We simulate case-control samples for each SNP given an additive model. The simulation strategy follows the steps in Algorithm 2. Although this simulation strategy includes more parameters than the first one, it does not allow to control the true parameter \( \lambda \). The only true parameter which is known from the beginning of the simulation is \( \pi_0 = \frac{N_0}{N} \). Thus, in this simulation strategy, we are only able to measure the accuracy in estimating \( \bar{\pi}_0 \). However, since \( \hat{\lambda} \) in equation (9) directly depends on the value of \( \bar{\pi}_0 \), a perfect estimate of \( \bar{\pi}_0 \) would automatically lead to a reliable estimate of \( \lambda \).

Following the above simulation algorithm, we conducted different simulations with different parameters. We took \( r = 1000, s = 1000, p = 0.2, v_0 = 0.01, OR_2 = 1.25, 1.5, 2, N_0 = 250, 500, 1000 \), \( 9000, 9500, 9750, N = 10000 \) and \( n = 100 \). Figure 3 represents the accuracy in estimating \( \bar{\pi}_0 \). From this figure we observe that the proposed estimator of \( \pi_0 \) using the MM approach has a very desirable performance. As discussed above, this convinces that the corresponding non-centrality parameter \( \lambda \) was also perfectly estimated. Consequently, the resulting LFDR estimates are highly precise and reliable. As observed from Figure 3, an increase in OR leads to a decreased MSE, as expected.
Algorithm 2 Second simulation strategy.

Step 1. Specify the numbers of cases \( r \) and controls \( s \), the allele frequency \( p \) for the risk allele \( B \) and the reference penetrance \( \nu_0 \).

Step 2. Take \( l = 1 \).

Step 3. Take \( OR_2 = 1 \).

Step 4. Calculate \( v_2 \) using the following equation

\[
v_2 = \frac{\exp(\beta_0 + \beta_2)}{1 + \exp(\beta_0 + \beta_2)},
\]

where \( \beta_0 = \log \left( \frac{\nu_0}{1 - \nu_0} \right) \) and \( \beta_2 = \log(OR_2) \) (the above equation is in fact the prospective logistic regression model).

Step 5. Calculate \( v_1 = \frac{1}{2}(v_0 + v_2) \) (this is due to selecting an additive model).

Step 6. Calculate \( k = \sum_{i=0}^{2} v_i g_i \), where \( g_0 = (1 - p)^2, g_1 = 2p(1 - p) \) and \( g_2 = p^2 \).

Step 7. For \( j = 1, 2, 3 \), calculate \( p_j = g_j v_j / k \) and \( q_j = g_j (1 - v_j) / (1 - k) \).

Step 8. Take \( m = 1 \).

Step 9. Generate random samples \( (r_0, r_1, s_2) \) and \( (s_0, s_1, s_2) \) independently from the multinomial distributions \( Mul(r; p_0, p_1, p_2) \) and \( Mul(s; q_0, q_1, q_2) \), respectively. This leads to a 2 \( \times \) 3 Table similar to Table 1.

Step 10. Similar to Table 2 construct the corresponding 2 \( \times \) 2 Table and compute the chi-square test statistic of independence, i.e.,

\[
x_t = \sum_{j=0}^{4} (o_j - e_j)^2 / e_j,
\]

where \( o_1 = 2r_0 + r_1, o_2 = r_1 + 2r_2, o_3 = 2s_0 + s_1, o_4 = s_1 + 2s_2, e_1 = 2R(2n_0 + n_1)/(2(R + S)), e_2 = 2R(n_1 + 2n_2)/(2(R + S)), e_3 = 2S(2n_0 + n_1)/(2(R + S)), e_4 = (2S(n_1 + 2n_2)/(2(R + S)) \) with \( R = r_0 + r_1 + r_2, S = s_0 + s_1 + s_2 \) and \( n_j = r_j + s_j, j = 1, 2, 3 \).

Step 11. Step up \( m \) by one and repeat Steps 9-10 until \( m = N_0 \).

Step 12. Take \( OR_2 = \gamma \neq 1 \) and repeat Steps 4-7.

Step 13. Increase \( m \) by one, and repeat Steps 9-10 until \( m = N \).

Step 14. Estimate \( \pi_0 \) and \( \lambda \) by \( \hat{\pi}_0 \) and \( \hat{\lambda} \) using the generated test statistics \( x_1, \ldots, x_N \) and equation (2).

Step 15. Compute the error of estimating the true proportion of unassociated SNPs by \( E_{\pi_0}^l = (\hat{\pi}_0 - \pi_0)^2 \), where \( \pi_0 = \frac{N_0}{N} \).

Step 16. Increase \( l \) by one and repeat Steps 3-15 for \( n \) times. Then, compute

\[
MSE_{\pi_0} = \frac{1}{n} \sum_{l=1}^{n} E_{\pi_0}^l.
\]

5 APPLICATIONS

5.1 Application to a microarray data set

In this subsection, we use a prostate data set used by Efron in which genetic expression levels for 6033 genes were obtained for 102 men including 50 normal control individuals and 52 prostate cancer patients. The interest is to test whether there is any difference between gene expression level and the prostate and normal individuals, \( i = 1, \ldots, 6033 \).

Let \( \bar{y}_i^{(1)} \) and \( \bar{y}_i^{(2)} \) be the mean of the normal individuals and cancer patients, and suppose that \( s_i \) is an estimate of the pooled sample standard error. To conduct this multiple hypothesis testing problem, the two-sample \( t \) test statistics \( t_i = \frac{\bar{y}_i^{(1)} - \bar{y}_i^{(2)}}{s_i} \) need to be computed first. One may then convert these test statistics to standard normal statistics \( z_i = \Phi^{-1}(F_{100}(t_i)) \) where \( \Phi \) and \( F_{100} \) are the cumulative distribution functions of normal and \( t \) distributions, respectively. By this transformation, the null hypothesis
can be expressed as \( H_0 : z_i \sim N(0, 1) \). Now, to apply our proposed estimation method, it suffices to use the transformation \( x_i = z_i^2 \). Then, the multiple hypothesis testing problem reduces to testing \( H_0 : x_i \sim \chi_1^2 \), and the resulting estimator form equation (9) reduces to \( \hat{\pi}_0 = 0.936 \). According to Efron’s HB approach and with the help of \textit{locfdr} package, \( \pi_0 \) is estimated to be equal to 0.932, see page 71. We also estimated \( \pi_0 \) using the ML approach of Padilla and Bickel. In this regard, we took \( c = 0 \) and \( d = 10 \) in equation (3), and applied the \textit{LFDR.MLE} package of Yang et al. \( \hat{\pi}_0 \) is estimated along with processing time on a personal computer (core i7, 3.5 GHz speed with 16 GB of RAM). Remarkably, the difference between estimates of \( \pi_0 \) using the HB and MM approaches is ignorable. We also observe that the MM approach is faster than the other approaches. It is almost 92 and 17 times faster than the ML and HB approaches, respectively.

| Estimation method | \( \hat{\pi}_0 \) | processing time (seconds) |
|-------------------|------------------|---------------------------|
| HB                | 0.932            | 0.069                     |
| ML                | 0.944            | 0.370                     |
| MM                | 0.936            | 0.004                     |

5.2 | Application to a comprehensive coronary artery disease data set

In this subsection, we apply the proposed LFDR estimation approach to analyze a comprehensive 1000 genomes-based genome-wide association data analyzed by Nikpay et. al. The data set consists of approximately 185,000 coronary artery disease (CAD) cases and controls, containing approximately 6.7 million variants with a minor allele frequency of greater than 0.05 and approximately 2.7 million variants with an allele frequency ranging between 0.005 and 0.05. The corresponding publicly available data consists of 9,455,777 SNPs with different information such as SNP name, chromosome name, effect allele, non-effect allele, frequency of effect allele, logistic regression coefficient (\( \hat{\beta} \)) with the corresponding standard deviation (\( \hat{\sigma}_\beta \)), and p-value. According to this data set, if p-value was a measure of association, then 2,213 total variants were significantly associated (p-value is less than \( 5 \times 10^{-8} \)). But here we are interested in verifying such association based on estimated LFDRs. To implement our proposed estimator, for each SNP \( i \), we took the test statistic \( x_i \) to be \( \left( \frac{\hat{\beta}}{\hat{\sigma}_\beta} \right)^2 \). Then, equation (9) with a processing time of 4.385 seconds led to \( \hat{\pi}_0 = 0.9967 \) and \( \hat{\lambda} = 21.9274 \). By these estimates, estimated LFDRs for the 2,213 variants had a maximum of 0.00032. This suggests that there is strong evidence for the association of the 2,213 SNPs with the CAD.
Nikpay et al. report ten new loci containing candidate causal genes newly implicating biological processes in vessel walls. Our analyses revealed that eight of the ten loci lead to estimated LFDRs less than 0.0003, but we are unable to confirm that the remaining two SNPs (rs11830157 and rs12976411) are associated with CAD.

We also applied the HB and ML approaches to analyze the data. The HB approach failed due to model misfit, and the ML approach with $c = 0$ and $d = 30$ in equation (5) led to a very close $\hat{\lambda}_0 (0.996)$ to the one reported by the MM approach. However, the processing time (445.237 seconds) was 339 times slower than the MM approach.

**6 DISCUSSION AND CONCLUDING REMARKS**

In this paper, we investigated estimating LFDRs for genetic association data. By reviewing well-known measures of association in the literature, we showed that many of the currently used measures reduce to a chi-square model with one degree of freedom. We presented a simple LFDR estimation strategy by using the MM estimators of the proportion $\pi_0$ and non-centrality parameter $\lambda$. The approach, as presented in Theorem 2 as well as Section 5, is simple and fast to apply. Also, as demonstrated by the two simulation strategies in Section 3 and the real data analysis in Section 5, it leads to reliable estimates. On the other hand, the ML approach of Padilla and Bickel highly depends on the bounds of $c$ and $d$ in (3), is time consuming, and the processing time increases with the number of SNPs as well as the length on the interval $[c, d]$. The HB approach of Efron also depends on some preset parameters such as the number of breaks in the discretization of the $z$-scores, the degrees of freedom for fitting the estimated density, etc., and it may fail due to model misfit.

Our proposed parametric method for estimating false discovery rates relies on the assumption that all non-null features have the same non-centrality parameter. This might not seem biologically realistic, but there are important advantages behind such an assumption. This assumption makes the estimation procedure easy and straightforward. Of course, having $N$ different non-centrality parameters in the model make it more biologically realistic, but that would rise the issue of interpretability. A single non-centrality parameter in the model is in fact a measure of the detectability of associations. It can also be interpreted as the average deviation of the data distributions of SNPs associated with a disease from the data distribution of those unassociated SNPs. It is also noteworthy that, according to the results presented in Section 5, having a single non-centrality parameter leads to an ideal model performance.

It is remarkable that, our proposed approach is similar to the classic hypothesis testing in the sense that the test statistic $x_i$ is compared to a threshold. However, the threshold in the MM approach is $h_u(\hat{\pi}_0, \hat{\lambda})$, while in the classic hypothesis testing it is just a $100(1 - \frac{\alpha}{2})\%$ quantile of the underlying distribution. This ideal property provides a more user-friendly estimator of LFDR than the other existing approaches.

It is worth adding that, estimating LFDRs in the literature is usually done by using some algorithms without knowing explicit form of estimators of the underlying parameters. For example, in the ML estimation used by Padilla and Bickel, an algorithm is applied to find arguments that maximize the likelihood function numerically, without providing any closed form of the resulting estimators of the parameter. Such algorithms may also require some unrealistic assumptions such as independency. On the contrary, our proposed approach offers explicit forms of estimators of the parameters $\pi_0$ and $\lambda$, without imposing any restriction to the model. This leads to user-friendly estimators using the simple Bayes rule provided in equation (11). All a user needs is the test statistics and estimated values of $\pi_0$ and $\lambda$.

As discussed in our second real data analysis, the estimated value of $\pi_0$ using our proposed approach is very close to the one reported in Efron. Obviously, this compliance confirms that the two approaches estimate $\pi_0$ very well, but this does not mean that the same threshold of estimated LFDR should be used. This is due to the fact that Efron’s LFDR estimation is based on the normal model while our proposed approach is on the basis of the chi-square model. Thus, if one is interested in using a 0.20 threshold when using Efron’s HB approach, she/he might use a different one (maybe 0.1 or less) when applying our proposed approach.

The estimation procedure presented in this paper can be used for other purposes, too. For example, Karimnezhad and Bickel introduce LFDR estimation in presence of some additional information such as genetic annotations. They use the ML approach of Padilla and Bickel but one may be interested in applying our proposed estimation approach in their reference class problem. If so, new estimators of LFDR will be derived.
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Conflict of interest

The authors declare no potential conflict of interests.