Robust Methods for Disease–Genotype Association in Genetic Association Studies: Calculate $P$-values Using Exact Conditional Enumeration instead of Asymptotic Approximations

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Abstract

Within the field of genetic association studies, detecting disease–genotype associations is a primary goal. For most diseases, the underlying genetic model is unknown, and we study seven robust test statistics for monotone disease–genotype association. For a given test statistic there are many ways to calculate a $p$-value, but in genetic association studies, $p$-value calculations have predominantly been based on asymptotic approximations and on simulated permutation. We show that when the number of permutations tends to infinity, the permutation $p$-value approaches the exact conditional enumeration $p$-value, and further that calculating the exact conditional enumeration $p$-value is much more efficient than performing simulated permutations. We then answer two research questions. (i) Which of the seven robust test statistics under study are the most powerful for monotone genetic models? (ii) Based on test size, power, and computational considerations, should asymptotic approximations or exact conditional enumeration be used for calculating $p$-values? We have studied case–control sample sizes with 500–5000 cases and 500–15000 controls, and significance levels from $5 \cdot 10^{-8}$ to 0.05, thus our results are applicable to genetic association studies with only one genetic marker under study, intermediate follow-up studies, and genome wide association studies. Our main findings are as follows. If all monotone genetic models are of interest, the best performance in the situations under study is achieved for the robust test statistics based on the maximum over a range of Cochrane–Armitage trend tests with different scores and for the constrained likelihood ratio test. For significance levels below 0.05, for the test statistics under study, asymptotic approximations may give a test size up to 20 times the nominal level, and should therefore be used with caution. Further, calculating $p$-values based on exact conditional enumeration is a powerful, valid and computationally feasible approach, and we advocate its use in genetic association studies.

1 Introduction

In genetic association studies the aim is to detect a possible association between a phenotype and one or many genetic markers. This can be done for one marker at a time. We will consider biallelic genetic markers, giving three possible genotypes. For each genetic marker the following steps can be performed. (i) First an hypothesis test situation is specified. (ii) This guides the choice of a test statistic. (iii) Then a method to calculate a $p$-value is chosen. (iv) Finally, the calculated $p$-value is compared to a chosen significance level to arrive at a conclusion.

A hypothesis test situation may be formulated as “no association between the disease and the genetic marker” versus “association between the disease and the genetic marker”. In some
genetic studies, in particular for monogenic diseases, the effect of the disease allele on the disease phenotype may be known to follow a specific genetic model, such as dominant, recessive, additive, or multiplicative (e.g., Camp, 1997). The genetic model can be used as alternative hypothesis to construct a test statistic tailored to detect this type of disease–genotype association. For genome-wide association (GWA) studies the underlying genetic model of a disease allele is seldom known (e.g., Devlin and Roeder, 2004). Therefore, it is desirable to base the statistical inference on a test statistic that give high power over a wide range of genetic models. These types of test statistics are commonly referred to as robust test statistics (Freidlin et al., 2002).

We will restrict our attention to performance under what we will call monotone genetic models, under which the genetic effect of the heterozygous genotype lies between the two homozygous genotypes. This means that we are not considering overdominant models. The first research questions we want to address in this presentation is which of the available popular robust test statistics that are most suitable for use when the underlying genetic model is unknown, but monotone.

Two approximations that have been used to calculate p-values for disease–genotype association are asymptotic methods and simulated permutations (Sladek et al., 2007). We will argue that exact conditional enumeration yields the same p-values as permutation when the number of permutations tends to infinity, and is also less computationally intensive than simulated permutations. We will therefore consider asymptotic methods and exact conditional enumeration. Many other ways of calculating p-values exists, see Langaas and Bakke (2013) for a presentation of unconditional enumeration methods for discrete distributions suitable for small to moderate sample sizes.

Exact conditional enumeration is a general method by which the p-value of an outcome is calculated based on a test statistic and the conditional probabilities of all possible outcomes of the conditional experiment in question. The most popular use of exact conditional enumeration is the Fisher exact test. There is a large body of literature on hypothesis testing in \(2 \times 2\) contingency tables, where conditional tests are often found to be less powerful than unconditional alternatives, as described by Mehrotra et al. (2003) and Lydersen et al. (2009). Our focus is on disease–genotype association for a biallelic marker in a case–control setting, which means that the data can be presented in a \(2 \times 3\) contingency table. The conditioning is done on the column margins, and the conditional probability of an outcome is a trivariate hypergeometric probability. Due to the less discrete nature of higher order contingency tables, some researchers have found that the power disadvantage of the conditional test tends to be less pronounced than for the \(2 \times 2\) contingency table (Mehta and Hilton, 1993). For larger contingency tables exact conditional enumeration is believed to require substantial computer resources (enumeration and summation of probabilities) and thus not to be feasible for testing for disease–genotype association. We will show that even for large sample sizes, 5000 cases and at least 5000 controls in a \(2 \times 3\) contingency table, exact conditional enumeration can be performed in a fraction of a second on a standard computer. Turning to the asymptotic methods, it is known that asymptotic methods will not preserve test size in situations where the asymptotic approximation is poor. The second research question we want to address is therefore whether asymptotic or exact conditional enumeration methods are most suitable to use in terms of power, test size and computational resources when testing disease–genotype association with a robust test statistic.

In GWA studies multiple testing correction is commonly performed by controlling the familywise error rate by the Bonferroni method. When a single genetic marker is studied, a significance level of \(\alpha = 0.05\) is commonly used. For larger candidate studies, or follow-up studies, with 10–1000 genetic markers under study, significance levels of the order \(5 \cdot 10^{-3} – 5 \cdot 10^{-5}\) may be used. For genome-wide association (GWA) studies with ten thousand to one million genetics markers, significance levels \(5 \cdot 10^{-6} – 5 \cdot 10^{-8}\) have been used.
The expected frequencies of the control population. Let the genotype frequencies for the three genotypes in the population under study, \( n_1 \), \( n_2 \), and \( N \) be the disease prevalence, and \( k \) be the penetrance, i.e. the conditional probability of disease given genotype \( i \). Then \( p_i \) and \( q_i(1 - k) = (1 - f_i)g_i \) for \( i = 0, 1, 2 \). The null hypothesis that we will investigate is that the penetrances are equal for the three possible disease given genotype \( i \). Let  

### 2 Methods

#### 2.1 Notation and data

We will to some extent adopt the notation of Joo et al. (2009) and Langaas and Bakke (2013). We assume that genotypes and a dichotomous phenotype (disease) are collected in a case–control study, and that the genotype data are from biallelic genetic markers with alleles \( a \) and \( A \). For each genetic marker, we assume that \( A \) is the high risk allele, and index the three genotypes \( aa \), \( aA \), and \( AA \), by 0, 1, 2, respectively. Further, for each genetic marker let \( g_0 \), \( g_1 \) and \( g_2 \) be the genotype frequencies for the three genotypes in the population under study, \( p_0 \), \( p_1 \), and \( p_2 \) the genotype frequencies of the case population (disease phenotype) and \( q_0 \), \( q_1 \), and \( q_2 \) the genotype frequencies of the control population.

For each genetic marker we may present the collected data in a 2 × 3 contingency table (Table 1). The number of cases and controls with genotype \( i \) by \( m_i = x_i + y_i \), \( i = 0, 1, 2 \). Let \( m_1 = x_0 + x_1 + x_2 \) denote the total number of cases, \( m_2 = y_0 + y_1 + y_2 \) the total number of controls, and let \( N = n_1 + n_2 = m_0 + m_1 + m_2 \).

The presentation is organized as follows. In Section 2 we present notation, data structure, test statistics, methods for calculating \( p \)-values and estimation of power. We then present the results of a large simulation study to compare the power of different robust test statistics combined with either the asymptotic method or the exact conditional enumeration method in Section 3. We discuss in Section 4 and conclude in Section 5.

### Table 1: Notation for 2 × 3 table, case–control data.

| Genotype | \(aa\) | \(aA\) | \(AA\) | Total |
|----------|-------|-------|-------|-------|
| Case     | \(x_0\) | \(x_1\) | \(x_2\) | \(n_1\) |
| Control  | \(y_0\) | \(y_1\) | \(y_2\) | \(n_2\) |
| Total    | \(m_0\) | \(m_1\) | \(m_2\) | \(N\) |

(The Wellcome Trust Case Control Consortium 2007). Dudbridge and Gusnanto (2008) advocated using significance level \( 7.2 \cdot 10^{-8} \) for general GWA studies. When we investigate our two research questions we will study significance levels in the range \( 5 \cdot 10^{-2} - 5 \cdot 10^{-5} \), and sample sizes in the range 500–5000 cases and 500–15000 controls.

#### 2.2 Statistical Hypotheses and Genetic Models

Let \( k \) be the disease prevalence, and \( f_i \) be the penetrance, i.e. the conditional probability of disease given genotype \( i \). Then \( p_i k = f_i g_i \) and \( q_i (1 - k) = (1 - f_i) g_i \) for \( i = 0, 1, 2 \). The null hypothesis that we will investigate is that the penetrances are equal for the three possible...
genotypes,

\[ f_0 = f_1 = f_2, \tag{2} \]

which can be shown to be equivalent to \( p_i = q_i \) for \( i = 0, 1, 2 \). Further, denote by \( \lambda_1 = f_1/f_0 \) and \( \lambda_2 = f_2/f_0 \) the genotype relative risks. We define a monotone genetic model to satisfy

\[ f_0 \leq f_1 \leq f_2, \tag{3} \]

or alternatively \( 1 \leq \lambda_1 \leq \lambda_2 \), which can be shown to be equivalent to \( p_0/q_0 \leq p_1/q_1 \leq p_2/q_2 \). As alternative hypotheses we consider monotone genetic models where at least one of the equalities are strict. Those models can be parameterized by

\[ \lambda_1 = 1 - \delta + \delta \lambda_2, \tag{4} \]

where \( \lambda_2 > 1 \) and \( 0 \leq \delta \leq 1 \). The value \( \delta = 0 \) yields the recessive genetic model, \( \delta = 1/2 \) the additive genetic model, and \( \delta = 1 \) the dominant genetic model. We will refer to the genetic model with \( \delta = 1/4 \) as semi-recessive and \( \delta = 3/4 \) as semi-dominant.

2.3 Test Statistics and Asymptotic Distributions

We now consider test statistics for testing the null hypothesis (2) against the alternative of a general monotone genetic model (4) or some specified monotone genetic model. The potential high risk allele is often unknown. Therefore all tests will be two-sided, in the sense that the conclusion of the test will be the same if the data for each homozygote are swapped.

2.3.1 Cochran–Armitage Trend Test (CATT)

The Cochran–Armitage test for trend (CATT) \cite{Armitage, 1955; Cochran, 1954; Sasieni, 1997; Slager and Schaid, 2001} is often used to test the null hypothesis (2) against one of the common (recessive, additive, dominant) genetic models (alternative hypotheses) in (4). It is based on the statistic \( \sum_{i=0}^{2} s_i (x_i/n_1 - y_i/n_2) \), where \( s_0, s_1, s_2 \) are scores appropriate for the alternative hypothesis in question. Standardizing and replacing unknown parameters \( p_i, q_i \) by estimators \( m_i/N \), we obtain the CATT test statistic,

\[ \text{CATT} = \frac{\sum_{i=0}^{2} s_i (n_2 x_i - n_1 y_i)}{\sqrt{n_1 n_2 \left( \sum_{i=0}^{2} s_i^2 m_i - \frac{1}{N} \left( \sum_{i=0}^{2} s_i m_i \right)^2 \right)}} \]

which asymptotically has a standard normal distribution under the null hypothesis. The absolute value of CATT is invariant to linear transformations of the scores, so they are chosen \( (s_0, s_1, s_2) = (0, s, 1) \), and we use the notation \( \text{CATT}_s \) for CATT with those scores. The value of \( s \) is chosen as \( s = 0, 1/2, 1 \) for the recessive, additive and dominant model of (4), respectively \cite{Zheng et al., 2003}. The index \( s \) thus denotes which genetic model (alternative hypothesis) is used. A large value of \( |\text{CATT}_s| \) indicates rejection of the null hypothesis.

We will study the \( \text{CATT}_{1/2} \) test statistics further.

2.3.2 Pearson Chi-Squared Test (Pearson)

The well-known Pearson chi-squared test statistic

\[ \sum_{i=0}^{2} \left( \frac{(x_i - m_i n_1/N)^2}{m_i n_1/N} + \frac{(y_i - m_i n_2/N)^2}{m_i n_2/N} \right) \]
is not tailored to be powerful for monotone genetic models (3) in particular but rather to test against the more general alternative that \( f_0, f_1 \) and \( f_2 \) are not all equal. Under the null hypothesis, the Pearson test statistic for our \( 2 \times 3 \) situation asymptotically follows a chi-squared distribution with two degrees of freedom. A large value indicates rejection of the null hypothesis.

### 2.3.3 Minimum \( p \)-Value Test (MIN2)

The statistic \( \text{MIN2} \) is defined as the minimum of the asymptotic \( p \)-values of \( \text{CATT}_{1/2} \) and of the Pearson chi-squared statistic. It is not a valid \( p \)-value itself, but its asymptotic distribution under the null hypothesis is given by

\[
\Pr(\text{MIN2} \leq t) \to \frac{1}{2} t + \frac{1}{2} e^{-q/2} - \frac{1}{2\pi} \int_{q}^{\ln t} e^{-v/2} \arcsin \left( \frac{2q}{v} - 1 \right) dv,
\]

where \( q \) is the \( 1-t \) quantile of the chi-squared distribution with one degree of freedom (Joo et al., 2009). A small value of \( \text{MIN2} \) indicates rejection of the null hypothesis.

### 2.3.4 Maximum Test (MAX3)

The statistic \( \text{MAX3} = \max(\left| \text{CATT}_0 \right|, \left| \text{CATT}_{1/2} \right|, \left| \text{CATT}_1 \right|) \) was proposed as an alternative to \( \text{CATT} \) when the genetic model is unknown but monotone, with emphasis on the recessive, additive or dominant model (Freidlin et al., 2002). Asymptotically, \( \text{CATT}_{1/2} \) is a linear combination of \( \text{CATT}_0 \) and \( \text{CATT}_1 \), and \( (\text{CATT}_0, \text{CATT}_1) \) has a bivariate normal asymptotic distribution (Zang et al., 2010). The asymptotic \( p \)-value of \( \text{MAX3} \) can be found as the probability of a bivariate normal pair lying outside a region, which is in general hexagonal, in the plane. Specifically,

\[
\Pr(\text{MAX3} \geq t) \to 1 - 2 \int_{0}^{(1 - \omega_1)t/\omega_0} \phi(x) \left( \Phi \left( \frac{t - \rho x}{\sqrt{1 - \rho^2}} \right) - \Phi \left( \frac{-t - \rho x}{\sqrt{1 - \rho^2}} \right) \right) dx \\
- 2 \int_{(1 - \omega_1)t/\omega_0}^{t} \phi(x) \left( \Phi \left( \frac{(t - \omega_0 x)/\omega_1 - \rho x}{\sqrt{1 - \rho^2}} \right) - \Phi \left( \frac{-t - \rho x}{\sqrt{1 - \rho^2}} \right) \right) dx,
\]

where

\[
\omega_0 = \sqrt{\frac{g_2(1 - g_2)}{g_0(1 - g_0) + g_2(1 - g_2) + 2g_0g_2}} \quad \text{and} \quad \omega_1 = \sqrt{\frac{g_0(1 - g_0)}{g_0(1 - g_0) + g_2(1 - g_2) + 2g_0g_2}}
\]

are the coefficients making \( \text{CATT}_{1/2} \to \omega_0 \text{CATT}_0 + \omega_1 \text{CATT}_1 \) asymptotically,

\[
\rho = \sqrt{\frac{g_0g_2}{(1 - g_0)(1 - g_2)}} \quad (5)
\]

is the asymptotic correlation coefficient of \( \text{CATT}_0 \) and \( \text{CATT}_1 \) under the null hypothesis (2), and \( \phi \) and \( \Phi \) are the standard normal pdf and cdf, respectively (Zang et al., 2010). When the asymptotic \( p \)-value is computed, \( g_0, g_1 \) and \( g_2 \) must be replaced by their consistent estimators \( m_0/N, m_1/N \) and \( m_2/N \), respectively.

### 2.3.5 Constrained Maximum Test (CMAX)

The Pearson chi-squared test statistic (Section 2.3.2) is equal to \( \text{CATT}_2^2 \), where the score is determined by the data, \( s = (x_1/m_1 - x_0/m_0)/(x_2/m_2 - x_0/m_0) \) (Zheng et al., 2009), which
Similarly, under the constraint of a dominant model, the maximum is obtained at \( f = \frac{m_0}{m_0 + m_1} \left( \frac{x_0 + x_1}{n_1}, \frac{y_0 + y_1}{n_2} \right) \), \( i = 0, 1 \) and \( p_2 = \frac{x_2}{n_1}, q_2 = \frac{y_2}{n_2} \), which gives the maximum

\[
l_{\text{rec}} = (x_0 + x_1) \ln(x_0 + x_1) + x_2 \ln x_2 + (y_0 + y_1) \ln(y_0 + y_1) + y_2 \ln y_2 + m_0 \ln m_0 + m_1 \ln m_1 - (m_0 + m_1) \ln(m_0 + m_1) - n_1 \ln n_1 - n_2 \ln n_2.
\]

Similarly, under the constraint of a recessive model, \( f_1 = f_2 \), or equivalently, \( p_1/q_1 = p_2/q_2 \), the maximum is obtained at

\[
p_0 = \frac{x_0}{n_1}, \quad q_0 = \frac{y_0}{n_2} \quad \text{and} \quad \left( p_i, q_i \right) = \frac{m_i}{m_1 + m_2} \left( \frac{x_1 + x_2}{n_1}, \frac{y_1 + y_2}{n_2} \right), \quad i = 1, 2,
\]
which gives the maximum

\[
l_{\text{dom}} = x_0 \ln x_0 + (x_1 + x_2) \ln(x_1 + x_2) + y_0 \ln y_0 + (y_1 + y_2) \ln(y_1 + y_2)
+ m_1 \ln m_1 + m_2 \ln m_2 - (m_1 + m_2) \ln(m_1 + m_2) - n_1 \ln n_1 - n_2 \ln n_2.
\]

Under the null hypothesis \( q \), the maximum is obtained at \( p_i = q_i = m_i/N \), giving the maximum

\[
l_0 = m_0 \ln m_0 + m_1 \ln m_1 + m_2 \ln m_2 - N \ln N.
\]

Then

\[
\text{CLRT} = \begin{cases} -2(l_1 - l_0) & \text{if } 0 \leq s \leq 1 \\ -2(\max(l_{\text{rec}}, l_{\text{dom}}) - l_0) & \text{otherwise}, \end{cases}
\]

where \( s \) is the data-driven score defined in Section 2.4.3. This is the same statistic as obtained by Wang and Sheffield (2005), who showed that CLRT has the same asymptotic distribution under the null hypothesis as described for CMAX (Section 2.3.3).

### 2.3.7 Maximin Efficiency Robust Test (MERT)

A maximin efficiency robust test (Gastwirth, 1985) can be constructed from CATT₂.3.7 Maximin Efficiency Robust Test (MERT) under the null hypothesis as described for CMAX (Section 2.3.5).

A maximin efficiency robust test \( \text{Gastwirth, 1985} \) can be constructed from CATT₀ and CATT₁, giving MERT = \((\text{CATT}_0 + \text{CATT}_1)/\sqrt{2(1 + \rho)}\), where \( \rho \) is defined in (5) (Zheng et al., 2006). It has an asymptotic standard normal distribution under the null hypothesis. A large value of \(|\text{MERT}|\) indicates rejection of the null hypothesis.

### 2.4 Using Conditional Enumeration to Calculate \( p \)-Values

When an outcome \( z = (x_0, x_1, x_2, y_0, y_1, y_2) \), is presented as a contingency table (Table 1) the column margins are \( m_0 = x_0 + y_0 \), \( m_1 = x_1 + y_1 \) and \( m_2 = x_2 + y_2 \). When we condition on the column margins \( M(z) = (m_0, m_1, m_2) \), the probability under the null hypothesis of an outcome \( z \) is a trivariate hypergeometric probability

\[
\Pr(Z = z \mid M(Z) = M(z)) = \frac{(m_0 \choose x_0)(m_1 \choose x_1)(m_2 \choose x_2)}{(N \choose n_1)},
\]

(6)

showing that the column margins are sufficient statistics for the genotype frequencies, which would otherwise be nuisance parameters. Any test statistic \( T \) (with, say, large values indicating rejection of the null hypothesis) defines a \( p \)-value of an outcome \( z \) conditioned on its column margins \( M(z) \). It can be calculated by the sum

\[
p(z) = \Pr(T(Z) \geq T(z) \mid M(Z) = M(z)) = \sum_{T(z') \geq T(z)} \Pr(Z = z' \mid M(z') = M(z)).
\]

(7)

The number of summands in (7) is much smaller than it would have been without conditioning, making summation also feasible for relatively large studies. Bakke and Langaas (2012) found a formula for the maximum number of summands in (7). For unbalanced sample sizes where \( n_2 \geq 2n_1 \) the maximum number of summands is simply \((n_1 + 2)^2\). Numerical examples are presented in Table 2.

We have seen that the outcome of an experiment can be presented as a contingency table \( z = (x_0, x_1, x_2, y_0, y_1, y_2) \). The outcome may alternatively be given on the individual level as two vectors of length \( N \), one giving the disease status and one giving genotype status. Thus, entry \( l \) in the disease vector gives the disease status of individual \( l \) and entry \( l \) in the genotype vector gives the coded genotype of individual \( l \). In permutation testing we generate \( b \) new
Table 2: Maximum number $N^*$ of tables with given column margins for sample size $n_1$ cases and $n_2$ controls. This will be the maximum number of summands when calculating the exact conditional enumeration $p$-value in Equation (7). The notation $\geq i$ means that $N^*$ is the same for all $n_2 \geq i$.

| $n_1$ | 500  | 500  | 1000 | 1000 | 5000 | 5000 |
|-------|------|------|------|------|------|------|
| $n_2$ | 500  | $\geq$1000 | 1000 | $\geq$2000 | 5000 | $\geq$10000 |
| $N^*$ | 83834 | 125751 | 334334 | 501501 | 8338334 | 12507501 |

outcomes of our experiment by permuting (shuffling) the genotypes vector, while keeping the disease vector fixed. This gives $b$ new contingency tables with the same margins as the observed contingency table. The permutation $p$-value is given as the proportion of the $b + 1$ outcomes (the original outcome and the $b$ permutation outcomes) having a value of the test statistic $T$ greater than or equal to that of the original outcome. The permutation $p$-value is valid (Phipson and Smyth, 2010). When $b$ tends to infinity the permutation $p$-value equals the exact conditional enumeration $p$-value. This can be seen by the fact that the permutation procedure is a trivariate hypergeometric experiment, drawing genotypes of the $n_1$ cases from the $m_0$, $m_1$, $m_2$ of each genotype. Moldovan and Langaas (2013) show in a worked-through example how to calculate the exact conditional enumeration $p$-value using the MAX3 test statistic.

We recommend using the exact conditional enumeration $p$-value, and not the simulated permutation $p$-value, based on the following arguments. If the permutation algorithm is run more than once for the same observed outcome, this may result in a different simulated permutation $p$-value for each run, which for a given significance level may lead to different hypothesis testing decisions. For GWA studies a significance level of $5 \cdot 10^{-8}$ is routinely used. To be able to arrive at a $p$-value below this significance level $b$ must at least be $2 \cdot 10^7$. Using permutation with very large values of $b$ is very inefficient compared to using (7) directly, as can also be seen from Table 2.

2.5 Validity of $p$-values

For a chosen significance level $\alpha$ and an outcome $z$, the null hypothesis is rejected if the $p$-value $p(z) \leq \alpha$. For a test to keep its size, the probability of rejection under the null hypothesis should be less than or equal to $\alpha$, i.e. $Pr(p(Z) \leq \alpha) \leq \alpha$ for all $\alpha$ and all parameters under the null hypothesis. Such a $p$-value is called valid (Casella and Berger, 2001, p. 397).

When calculating a $p$-value based on the asymptotic distribution of a test statistic, there is no reason to believe that this will be a valid $p$-value for the sample size under study. The conditional $p$-value defined in Section 2.4 is on the other hand always valid, not only considered as a $p$-value when the experiment is conditioned on $M(Z)$, but also considered as a $p$-value for the original experiment (here case–control) (Casella and Berger, 2001, p. 399).

2.6 Power

Desirable properties of a $p$-value are validity and high power (the probability to reject $H_0$). If the sample space is discrete, the power at a parameter vector $\theta$ of a test defined by $p(Z)$ for a given $\alpha$ is

$$\gamma(\theta) = Pr_{\theta}(p(Z) \leq \alpha) = \sum_{p(z) \leq \alpha} Pr_{\theta}(Z = z),$$

(8)
where the probabilities depend on the parameter vector \( \theta \), and the summation is over all outcomes \( z \) having a \( p \)-value not greater than \( \alpha \). The test size is \( \text{sup}_{\theta \in \Theta_0} \gamma(\theta) \), where the supremum is taken over all parameter vectors under the null hypothesis.

In our set-up, explained in detail in Section 2.3, the number of summands in \( \gamma \) is maximally \( (n_1 + 2)(n_2 + 2) \), which becomes too large for practical use for the sample sizes we consider. We instead estimate test size and power using simulation. We base our calculations on \( b \) independent random draws from the probability distribution for the data \( \mathcal{P} \). Let \( p(z_i) \) be the calculated \( p \)-value for drawing \( i \). Then the estimated power, \( \hat{\gamma}(\theta) \), is

\[
\hat{\gamma}(\theta) = \frac{1}{b} \sum_{i=1}^{b} I_{[0, \alpha]}(p(z_i)),
\]

where \( I \) is the indicator function having value 1 if \( p(z_i) \leq \alpha \) and 0 otherwise. That is, the power (or test size) is estimated as the fraction of \( p \)-values below \( \alpha \) for the \( b \) independent random draws from the parameter vector \( \theta \).

3 A study on test size and power

We will now investigate size and power using \( p \)-values from the asymptotic approximations and the exact conditional enumeration for the seven statistics from Section 2.3 in various settings.

3.1 Set-up

In most genetic association studies the number \( n_1 \) of cases does not exceed the number \( n_2 \) of controls. We will also make this assumption. We consider both balanced and unbalanced designs. For \( n_1 \) equal to 500 or 1000, we consider \( n_2 \) to be 1, 2, 3, 4, 5 times \( n_1 \). For \( n_1 = 5000 \), we consider \( n_2 \) to be 1, 2, 3 times \( n_1 \). This gives in total 13 sample sizes \((n_1, n_2)\) to consider.

Our data generation procedure is inspired by Joo et al. (2009). We have studied a disease prevalence of 10%, since genetic association studies in general are designed to target common diseases. Most GWA studies are based on single nucleotide polymorphisms (SNPs) with minor allele frequency (MAF) at least 5%. We have chosen a MAF of 10%, and we only consider populations under Hardy–Weinberg equilibrium, which gives genotype frequencies \( g_0 = (1 - \text{MAF})^2 = 0.81 \), \( g_1 = 2\text{MAF}(1 - \text{MAF}) = 0.18 \), \( g_2 = \text{MAF}^2 = 0.01 \). We assume that the minor allele is also the disease allele. For each situation under study we calculate \( \theta = (p_0, p_1, p_2, q_0, q_1, q_2) \) based on the formulas in Section 2.2 and draw data based on the probability distribution \( \mathcal{P} \).

Under the null hypothesis of equal penetrances for the genotypes, we draw \( 4 \cdot 10^8 \) samples from the study population. For this number of simulated samples we will for a valid test at significance level \( 5 \cdot 10^{-8} \) get an approximate 95% confidence interval for the test size that have half-length \( 1.96 \cdot \sqrt{5 \cdot 10^{-8} \cdot (1 - 5 \cdot 10^{-8})/(4 \cdot 10^8)} = 7 \cdot 10^{-9} \).

We also study populations under alternative hypotheses, as presented in Section 2.2. Parameters chosen for the alternative hypotheses are the genotype relative risk \( \lambda_3 = 1.1, 1.2, 1.5, 2 \) and the genetic model parameter \( \delta = 0, 1/4, 1/2, 3/4, 1 \). The genotype relative risk \( \lambda_1 \) is calculated from \( \delta \) and \( \lambda_2 \) using (3).

For all the possible combinations for \( \lambda_2 \) and \( \delta \) (as given above), and for each of the 13 sample sizes we drew one million random samples from the population described by these parameter values. This resulted in \( 4 \cdot 5 \cdot 13 = 260 \) situations. With this number of samples we will for a true power of 0.8 achieve an approximate 95% confidence interval for the power with half-length \( 1.96 \cdot \sqrt{0.8 \cdot (1 - 0.80)/(1 \cdot 10^8)} = 8 \cdot 10^{-4} \).
For each sample drawn we calculated the asymptotic and the exact conditional enumeration
$p$-values for the seven test statistics (CATT\textsubscript{1/2}, Pearson, MIN2, MAX3, CMAX, CLRT and
MERT) under study, and estimated the test size or power as the relative number of $p$-values
falling below the significance levels investigated.

For evaluation of test size and power we present results for significance levels $\alpha$ in the range
$5 \cdot 10^{-2}$ to $5 \cdot 10^{-8}$.

### 3.2 Computational details

The numerical calculations were performed in the C++ language of the GNU Compiler Collection
4.4.3. For generating multinomial vectors, making statistical distribution calculations and
numerical integration, the GNU Scientific Library 1.13 was used. To reduce computation time,
the parallel language extension OpenMP was used to distribute the generation of tables and
subsequent statistical calculations among several threads operating on different processors.

For the asymptotic method, power calculations in the case of CATT\textsubscript{1/2}, Pearson, MIN2 and
MERT were done by comparing test statistics for simulated data with critical values, which is
faster than calculating $p$-values explicitly. In the case of MAX3, CMAX and CLRT, $p$-values
depend on estimated parameters, and had to be calculated for each simulated table.

For the exact conditional enumeration method, to avoid numerical overflow, the hypergeo-
metric probabilities (6) were calculated by adding logarithms of factorials and then taking the
antilogarithm. To gain speed, the $\ln l!$ were computed once and tabulated for $l = 0, 1, \ldots, N$.
If, for a simulated table $(x_0, x_1, x_2, y_0, y_1, y_2)$, during the evaluation of the exact conditional
enumeration $p$-value (7), the sum for all seven statistics $T$ had exceeded the highest significance
level considered, 0.05, the evaluation was aborted, since the table would then not contribute to
the power. Also, to speed up the time for a possible early abortion of the summation (7), the
summation was started at tables potentially having a high conditional probability (6), namely
those having $x_0$ as the upper left entry. Then tables having $x_0 + 1$ as the upper left entry
were considered and so on upwards, and thereafter the process was repeated going from $x_0 - 1$
downwards.

For the largest sample size considered in the simulation study, $(5000, 15000)$, the maximum
number of summands in the calculation of the exact conditional enumerations $p$-value is 12.5
million. This maximum number will occur for tables having equal or nearly equal column mar-
gins. However, our choice of MAF = 0.1 will with very low probability give such balanced column
margins. On a 4 $\times$ 6-core Xeon 2.67 GHz computer (Intel CPU) running Linux (Ubuntu 10.4),
using one tread, the computation of exact conditional enumeration $p$-values for 1000 tables with
sample size $n_1 = 5000$ and $n_2 = 15000$ drawn from the null hypothesis took under 14 seconds.
The corresponding computation for 1000 such tables drawn under an alternative hypothesis
having power near 100\% for all test statistics, took under two minutes. Calculation of the exact
conditional enumeration $p$-value is faster when tables are drawn from the true null hypothesis
than when tables are drawn from the alternative hypothesis. This is due to the fact that $p$-value
calculated for tables generated under the true null are in general larger than $p$-values for tables
generated under the alternative hypothesis, and that we abort the calculations when the $p$-value
exceeds 0.05.

For smaller sample sizes, computations are much faster. For $n_1 = 1000$, $n_2 = 1000$ the
timings are 2 seconds for the null hypothesis and 0.3 seconds for the alternative.

### 3.3 Effect of significance level and sample size

In Section 2.5 we pointed out that there is no guarantee that asymptotic methods preserves the
test size, while the exact conditional methods are always valid by construction. However, for
\( \alpha = 0.05 \) we found that the asymptotic methods for nearly all test statistics (except CLRT) kept the test size for all sample sizes investigated. With the exception of CLRT we get an increasing degree of mismatch between the observed and the nominal level for the asymptotic methods when \( \alpha \) decreases to \( 5 \cdot 10^{-8} \). Worst are MAX3 and CMAX for small and unbalanced designs, with test size up to 20 times the nominal level. For low significance levels, MAX3, Pearson, MIN2, MERT and CMAX keeps under 1.2 times the nominal level only for balanced designs and for designs having the number of controls twice the number of cases. CATT\(_{1/2}\) fares a little better, but at worst has size twice the nominal level, and CLRT is always within 1.5 times the level. In Table 3 we present estimated test sizes for balanced sample sizes for the asymptotic and exact conditional methods for all seven test statistics for three values of the significance level (low, \( 5 \cdot 10^{-2} \); intermediate, \( 5 \cdot 10^{-5} \); high, \( 5 \cdot 10^{-8} \)). To emphasize the need to use exact conditional methods instead of asymptotic methods for low significance levels and unbalanced sample sizes Table 4 gives test sizes for significance level \( 5 \cdot 10^{-8} \) for the asymptotic method.

Turning to the power study, we find that the power increases with increasing sample sizes and with increasing significance levels. This can be seen in Table 5 (the effect of significance level) and 6 (the effect of sample size). Keeping the total sample size fixed the highest power is observed for balanced sample sizes and decreases with increasing degree of unbalance. This can be observed (Table 6) for the sample size combinations \((n_1, n_2) = (500, 1500)\) and \((1000, 1000)\), both giving total sample size 2000, and this is also the case for \((500, 2500)\) and \((1000, 2000)\), with total sample size 3000.

3.4 Effect of genetic model

Since the asymptotic methods, with CLRT as the exception, are in general not valid, we base our discussion on comparing power based on the exact conditional enumeration \( p \)-values. But, the power of the invalid asymptotic methods will in general not be substantially larger than the power of the valid exact conditional methods, which is seen in Tables 5–8.

We have chosen to only study monotone genetic models, and we find that the effect of genetic model seem to be similar for all sample sizes and significance levels. Results for \((n_1, n_2) = (5000, 15000)\) and \(\alpha = 5 \cdot 10^{-6}\) are shown in Table 7.

For the recessive model \((\delta = 0)\) the CMAX and MAX3 methods performs the best (with very similar powers). The CATT\(_{1/2}\) performs poorly for the recessive model, as compared to all the other test statistics studied. The most extreme situation observed was for sample size \((5000, 15000)\) for \(\lambda_2 = 2\) and \(\alpha = 5 \cdot 10^{-6}\), where CATT\(_{1/2}\) gives a power of 4.6% while the CMAX gives a power of 88.4% (Table 7, fourth row from the top).

For the semi-recessive model \((\delta = 1/4)\) MERT gives the best performance (Table 7, rows 5–8).

For the additive model \((\delta = 1/2)\) the CATT\(_{1/2}\) performs the best. This is also true for the semi-dominant \((\delta = 3/4)\) and the dominant \((\delta = 1)\) models. The MERT test statistic has for these three genetic models lower power than the other test statistics. The other test statistics (Pearson, MIN2, MAX3, CMAX and CLRT) have comparable powers, in most cases slightly lower than CATT\(_{1/2}\) and higher than MERT (Table 7 lower part; Table 5 for the additive model).

3.5 General findings

From the results of our power study we may divide the test statistics into four groups based on their overall performance. (i) The CATT\(_{1/2}\) has very good performance for all models other than the recessive, (ii) MERT performs well for the semi-recessive model, but else has a less good performance, (iii) the three test statistics MAX3, CMAX and CLRT have very similar
Table 3: Scaled test size for test statistics, methods (A asymptotic, C exact conditional), balanced sample sizes and selected significance levels $\alpha$. The test sizes shown are scaled by multiplying by $5/\alpha$, so that 5.00 will give an exact test. The test size estimate is based on $4 \cdot 10^9$ simulations, giving 95% confidence interval half-lengths of $6.8 \cdot 10^{-6}$, $2.2 \cdot 10^{-7}$, $6.9 \cdot 10^{-9}$ for true test sizes $5 \cdot 10^{-2}$, $5 \cdot 10^{-5}$, $5 \cdot 10^{-8}$, respectively

| $n_1$ | $n_2$ | $\alpha$ | CATT$_{1/2}$ | Pearson | MIN2 | MAX3 | CMAX | CLRT | MERT |
|-------|-------|-----------|--------------|---------|------|------|------|------|------|
|       |       |           | A  | C  | A  | C  | A  | C  | A  | C  | A  | C  | A  | C  |
| 500   | 500   | $5 \cdot 10^{-2}$ | 5.00 | 4.21 | 4.78 | 4.91 | 4.77 | 4.66 | 4.75 | 4.14 | 4.62 | 4.77 | 4.66 | 4.75 | 4.14 | 4.62 | 4.77 | 4.66 | 4.75 | 4.14 | 4.62 | 4.77 | 4.66 | 4.75 |
|       |       | $5 \cdot 10^{-5}$ | 4.53 | 3.66 | 2.28 | 4.82 | 3.24 | 4.13 | 2.61 | 4.20 | 2.04 | 4.64 | 5.38 | 4.59 | 2.08 | 4.46 |
|       |       | $5 \cdot 10^{-8}$ | 3.15 | 2.95 | 1.20 | 4.80 | 2.02 | 3.42 | 1.85 | 3.42 | 1.05 | 3.98 | 3.02 | 3.88 | 0.15 | 3.98 |
| 1000  | 1000  | $5 \cdot 10^{-2}$ | 5.00 | 4.42 | 4.89 | 4.96 | 4.90 | 4.76 | 4.85 | 4.39 | 4.64 | 4.80 | 5.00 | 4.80 | 4.98 | 4.91 |
|       |       | $5 \cdot 10^{-5}$ | 4.76 | 4.02 | 3.06 | 4.96 | 3.73 | 4.48 | 3.10 | 4.45 | 2.75 | 4.87 | 6.19 | 4.70 | 3.36 | 4.80 |
|       |       | $5 \cdot 10^{-8}$ | 4.83 | 4.35 | 1.92 | 5.22 | 3.20 | 4.67 | 2.77 | 4.62 | 1.77 | 5.10 | 6.42 | 4.45 | 1.07 | 4.85 |
| 5000  | 5000  | $5 \cdot 10^{-2}$ | 5.00 | 4.73 | 4.98 | 4.99 | 4.98 | 4.90 | 4.97 | 4.71 | 4.76 | 4.93 | 4.81 | 4.93 | 5.00 | 4.98 |
|       |       | $5 \cdot 10^{-5}$ | 4.95 | 4.53 | 4.52 | 4.99 | 4.68 | 4.82 | 4.57 | 4.58 | 4.29 | 4.95 | 4.95 | 4.92 | 4.65 | 4.96 |
|       |       | $5 \cdot 10^{-8}$ | 4.53 | 3.80 | 4.10 | 5.03 | 3.70 | 4.05 | 3.58 | 4.33 | 3.20 | 4.67 | 4.95 | 4.70 | 4.12 | 5.00 |
Table 4: Scaled test size for test statistics, balanced and unbalanced sample sizes for the asymptotic method for significance level $\alpha = 5 \cdot 10^{-7}$. The test sizes shown are scaled by multiplying by $5/\alpha$, so that 5.00 will give an exact test. $4 \cdot 10^9$ simulations are run, giving 95% confidence interval half-lengths of $2.2 \cdot 10^{-8}$ for true test sizes $5 \cdot 10^{-7}$.

| $n_1$ | $n_2$ | CATT$_{1/2}$ | Pearson | MIN2 | MAX3 | CMAX | CLRT | MERT |
|-------|-------|--------------|---------|------|------|------|------|------|
| 500   | 500   | 3.81         | 1.61    | 2.55 | 2.28 | 1.50 | 3.66 | 0.54 |
| 1000  | 500   | 5.14         | 4.02    | 4.37 | 4.05 | 4.20 | 5.05 | 4.57 |
| 1500  | 500   | 6.01         | 14.81   | 11.78| 17.52| 15.12| 4.32 | 11.80|
| 2000  | 500   | 6.89         | 28.76   | 21.76| 33.46| 32.90| 3.99 | 18.44|
| 2500  | 500   | 7.60         | 42.75   | 31.05| 50.29| 46.66| 4.01 | 23.97|
| 1000  | 1000  | 4.55         | 2.19    | 3.30 | 2.71 | 1.95 | 6.83 | 1.78 |
| 2000  | 1000  | 5.15         | 5.76    | 5.46 | 6.12 | 5.75 | 5.97 | 5.46 |
| 3000  | 1000  | 5.35         | 11.84   | 9.54 | 12.33| 12.82| 5.31 | 9.04 |
| 4000  | 1000  | 5.89         | 17.95   | 13.99| 21.49| 20.25| 5.14 | 12.17|
| 5000  | 1000  | 6.22         | 23.74   | 17.95| 26.95| 26.23| 5.13 | 14.90|
| 5000  | 5000  | 4.85         | 4.01    | 4.29 | 4.06 | 3.75 | 5.05 | 4.13 |
| 10000 | 5000  | 5.10         | 5.21    | 5.18 | 5.45 | 5.42 | 4.97 | 5.31 |
| 15000 | 5000  | 5.23         | 6.84    | 6.30 | 7.27 | 7.23 | 5.11 | 6.21 |

performance and give good results for all genetic models, and lastly, (iv) Pearson and MIN2 also have very similar performance, in general slightly less powerful than the previous group, but is also known to work well for non-monotone genetic models (Joo et al., 2009).

In Table 5 we present powers for $\alpha = 5 \cdot 10^{-8}$ for all the sample sizes under study. For each sample size we have chosen the genetic model and effect size that give power (over all test statistics) closest to 80%. These results are influenced by a selection bias due to the fact that for small sample sizes only the dominant models with large effects sizes will achieve power near 80%, and for large sample sizes power near 80% will be achieved for additive to recessive models. Taking this into mind, we see that the general results presented above are reflected in this table. To summarize, the exact conditional enumeration method and the MAX3 test statistics is the most powerful for small balanced and slightly unbalanced sample sizes, (500, 500), (500, 1000) and (1000, 1000), for the dominant model. For (500, 1000), the MAX3 test statistics (exact conditional enumeration) gives 46.6 percentage points higher power than the MERT test statistic. For the unbalanced sample sizes, (500, 1500), (500, 2000), (500, 2500), (1000, 2000), (1000, 3000), (1000, 4000), (1000, 5000), the asymptotic method for the MAX3 test statistic is the most powerful for the dominant and semi-dominant model. This is not surprising since the asymptotic MAX3 shows large violations for these unbalanced sample sizes. For this reason, we do not recommend using the asymptotic method for these test statistics for unbalanced sample sizes. Only considering the exact conditional enumeration method for these unbalanced sample sizes, the best performance is found for the CATT$_{1/2}$ test statistics. For the largest sample sizes, MERT performs the best for the semi-recessive model for (5000, 5000), while the CATT$_{1/2}$ performs the best for the large unbalance sample sizes (5000, 10000), (5000, 15000) under the additive model.
Table 5: Power: Effect of test statistic, method (A asymptotic, C exact conditional) and significance level ($\alpha$). Sample size is $(n_1, n_2) = (5000, 5000)$, genetic model is additive ($\delta = 0.5$) and genetic relative risk $\lambda_2 = 1.5$.

| $\alpha$  | CATT$_{1/2}$ | Pearson | MIN2 | MAX3 | CMAX | CLRT | MERT |
|-----------|--------------|---------|------|------|------|------|------|
|           | A  | C  | A  | C  | A  | C  | A  | C  | A  | C  | A  | C  | A  | C  | A  | C  | A  | C  |
| 5 $\cdot$ 10^{-2} | 100.0 | 100.0 | 99.9 | 99.9 | 100.0 | 99.9 | 99.9 | 99.9 | 99.9 | 99.9 | 99.9 | 97.8 | 97.8 | 97.8 | 97.8 |
| 5 $\cdot$ 10^{-3} | 99.5 | 99.5 | 98.8 | 98.8 | 99.3 | 99.3 | 99.2 | 99.2 | 99.1 | 99.1 | 99.1 | 97.8 | 97.8 | 97.8 | 97.8 |
| 5 $\cdot$ 10^{-4} | 97.2 | 97.1 | 94.6 | 94.7 | 96.4 | 96.4 | 96.0 | 96.1 | 95.7 | 95.9 | 95.7 | 90.8 | 90.9 | 90.8 | 90.9 |
| 5 $\cdot$ 10^{-5} | 91.0 | 90.6 | 85.3 | 85.7 | 89.1 | 89.1 | 88.4 | 88.7 | 87.4 | 88.0 | 87.5 | 77.4 | 77.8 | 77.4 | 77.8 |
| 5 $\cdot$ 10^{-6} | 79.6 | 79.1 | 71.0 | 71.8 | 76.7 | 76.8 | 75.8 | 76.4 | 74.1 | 75.3 | 74.3 | 69.4 | 60.2 | 69.4 | 60.2 |
| 5 $\cdot$ 10^{-7} | 64.4 | 63.7 | 54.0 | 55.4 | 60.8 | 61.2 | 59.8 | 60.9 | 57.7 | 59.5 | 58.0 | 41.0 | 42.1 | 41.0 | 42.1 |
| 5 $\cdot$ 10^{-8} | 47.6 | 47.0 | 37.5 | 39.2 | 44.0 | 44.6 | 43.1 | 44.5 | 41.0 | 43.1 | 41.3 | 25.5 | 26.7 | 25.5 | 26.7 |
Table 6: Power: Effect of test statistic, method (A asymptotic, C exact conditional) and sample size ($n_1$ cases, $n_2$ controls). The genetic model is semi-dominant ($\delta = 3/4$), genetic relative risk $\lambda_2 = 1.5$ and significance level $\alpha = 5 \cdot 10^{-5}$.

| $n_1$ | $n_2$ | CATT$_{1/2}$ | Pearson | MIN2 | MAX3 | CMAX | CLRT | MERT |
|-------|-------|--------------|---------|------|------|------|------|------|
|       |       |              | A       | C    | A    | C    | A    | C    | A    | C    | A    | C    | A    | C    | A    | C    |
| 500   | 500   | 3.9          | 3.5     | 3.2  | 3.5  | 3.1  | 3.9  | 2.7  | 3.9  | 3.0  | 2.8  | 1.4  | 2.0  |
| 1000  | 1000  | 9.9          | 9.2     | 6.9  | 7.1  | 8.6  | 8.4  | 8.4  | 7.7  | 7.9  | 6.7  | 6.5  | 4.4  | 4.6  |
| 1500  | 1500  | 14.1         | 12.9    | 10.4 | 8.6  | 12.5 | 10.6 | 12.3 | 10.1 | 11.4 | 9.3  | 9.2  | 9.5  | 6.6  | 5.8  |
| 2000  | 2000  | 16.9         | 15.6    | 13.0 | 9.5  | 15.3 | 11.9 | 15.1 | 10.9 | 14.1 | 10.1 | 11.1 | 11.5 | 8.3  | 6.7  |
| 2500  | 2500  | 19.0         | 17.3    | 14.7 | 10.0 | 17.2 | 12.7 | 17.0 | 11.3 | 16.0 | 10.5 | 12.3 | 12.8 | 9.4  | 7.2  |
| 1000  | 1000  | 21.6         | 20.5    | 15.6 | 17.9 | 19.1 | 19.9 | 19.1 | 21.1 | 17.2 | 20.1 | 17.8 | 16.8 | 8.7  | 9.9  |
| 2000  | 2000  | 24.2         | 41.2    | 34.6 | 34.6 | 39.3 | 38.7 | 39.3 | 38.9 | 36.9 | 37.3 | 34.8 | 33.7 | 20.9 | 21.2 |
| 3000  | 3000  | 52.8         | 51.3    | 44.9 | 42.1 | 49.7 | 47.2 | 49.8 | 46.4 | 47.4 | 44.4 | 44.0 | 42.5 | 28.0 | 26.9 |
| 4000  | 4000  | 58.7         | 57.3    | 51.2 | 46.5 | 55.9 | 52.1 | 56.0 | 50.7 | 53.7 | 48.7 | 49.5 | 47.8 | 32.7 | 30.5 |
| 5000  | 5000  | 62.5         | 61.0    | 55.2 | 49.4 | 59.8 | 55.0 | 59.9 | 53.4 | 57.6 | 51.3 | 53.1 | 51.2 | 35.8 | 32.8 |
| 5000  | 5000  | 99.9         | 99.9    | 99.9 | 99.9 | 99.9 | 99.9 | 99.9 | 99.9 | 99.9 | 99.9 | 98.0 | 98.0 | 99.9 | 99.9 |
| 10000 | 10000 | 100.0        | 100.0   | 100.0| 100.0| 100.0| 100.0| 100.0| 100.0| 100.0| 100.0| 99.9 | 99.9 | 100.0| 100.0|
| 15000 | 15000 | 100.0        | 100.0   | 100.0| 100.0| 100.0| 100.0| 100.0| 100.0| 100.0| 100.0| 100.0| 100.0| 100.0| 100.0|
Table 7: Power: Effect of test statistic, method (A asymptotic, C exact conditional), genetic model ($\delta = 0$ recessive, $\delta = 1$ dominant) and genetic relative risk ($\lambda_2$). Sample size is $(n_1, n_2) = (5000, 15000)$ and significance level $\alpha = 5 \cdot 10^{-6}$.

| $\delta$ | $\lambda_2$ | CATT$_{1/2}$ | Pearson | MIN2 | MAX3 | CMAX | CLRT | MERT |
|----------|-------------|--------------|----------|------|------|------|------|------|
|          |             | A  | C  | A  | C  | A  | C  | A  | C  | A  | C  | A  | C  | A  | C  | A  | C  | A  | C  |
| 0        | 1.1         | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.25     | 1.1         | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.5      | 1.1         | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| 1        | 1.2         | 1.2 | 1.2 | 7.6 | 7.2 | 6.3 | 6.1 | 8.6 | 7.5 | 8.2 | 7.7 | 8.2 | 7.7 | 8.2 | 7.7 | 8.2 | 7.7 | 8.2 | 7.7 |
| 0.75     | 1.5         | 4.7 | 4.6 | 88.2 | 87.6 | 86.2 | 85.7 | 89.8 | 88.5 | 89.1 | 88.4 | 84.6 | 84.6 | 60.5 | 59.9 | 60.5 | 59.9 | 60.5 | 59.9 |
| 1        | 1.5         | 98.3 | 98.2 | 96.8 | 96.5 | 97.8 | 97.7 | 97.6 | 97.4 | 97.4 | 97.2 | 97.0 | 97.0 | 92.1 | 91.8 | 92.1 | 91.8 | 92.1 | 91.8 |
| 2        | 2           | 99.9 | 99.9 | 99.9 | 99.9 | 100.0 | 100.0 | 99.9 | 99.9 | 100.0 | 100.0 | 99.9 | 99.9 | 100.0 | 100.0 | 99.9 | 99.9 | 100.0 | 100.0 |
| 0.25     | 1.2         | 1.2 | 1.2 | 2.7 | 2.5 | 3.6 | 3.4 | 3.3 | 3.0 | 3.2 | 3.0 | 2.8 | 2.8 | 2.8 | 2.8 | 2.8 | 2.8 | 2.8 | 2.8 |
| 1        | 1.5         | 98.3 | 98.2 | 96.8 | 96.5 | 97.8 | 97.7 | 97.6 | 97.4 | 97.4 | 97.2 | 97.0 | 97.0 | 92.1 | 91.8 | 92.1 | 91.8 | 92.1 | 91.8 |
| 2        | 2           | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 0.75     | 1.1         | 0.6 | 0.5 | 0.3 | 0.3 | 0.5 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| 1        | 1.2         | 25.6 | 25.1 | 19.7 | 18.7 | 23.2 | 22.3 | 23.4 | 22.1 | 21.5 | 20.5 | 20.0 | 20.0 | 10.2 | 9.9 | 10.2 | 9.9 | 10.2 | 9.9 |
| 2        | 2           | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 1        | 1.1         | 2.3 | 2.2 | 1.6 | 1.5 | 2.0 | 1.9 | 2.1 | 1.8 | 1.7 | 1.6 | 1.5 | 1.5 | 0.7 | 0.6 | 0.7 | 0.6 | 0.7 | 0.6 |
| 1.2      | 1.2         | 64.7 | 64.1 | 59.9 | 58.5 | 63.2 | 62.0 | 64.6 | 62.9 | 61.6 | 60.2 | 59.7 | 59.8 | 27.7 | 27.1 | 27.7 | 27.1 | 27.7 | 27.1 |
| 1.5      | 1.5         | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 2        | 2           | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
Table 8: Power: Effect of test statistic, method (asymptotic, A, and exact conditional, C) and sample size ($n_1$ cases, $n_2$ controls) for significance level $\alpha = 5 \cdot 10^{-8}$. Values of the genetic model $\delta$ and $\lambda_2$ are chosen to give power closest to 80%. For each sample size the most powerful combination of method and test statistic is given in bold face, while the most powerful exact conditional enumeration method and test statistic is given in italic.

| $n_1$ | $n_2$ | $\delta$ | $\lambda_2$ | CATT$_{1/2}$ | Pearson | MIN2 | MAX3 | CMAX | CLRT | MERT |
|-------|-------|----------|-------------|---------------|----------|------|------|------|------|------|
|       |       |          |             | A    | C    | A    | C    | A    | C    | A    | C    |
| 500   | 500   | 1        | 2.0         | 36.8 | 36.0 | 32.3 | 39.2 | 35.3 | 38.0 | 37.5 | 41.2 | 34.2 | **41.4** | 36.0 | 37.3 | 7.5  | 14.0 |
| 1000  | 1     | 2.0      | 74.1        | 72.3 | 71.6 | 72.6 | 73.8 | 73.6 | 75.7 | **76.1** | 73.1 | 73.8 | 69.8 | 69.7 | 29.5 | 29.4 |
| 1500  | 1     | 2.0      | 86.2 | **83.8** | 84.9 | 78.2 | 86.2 | 80.9 | **87.6** | 82.1 | 85.8 | 79.0 | 81.9 | 82.7 | 43.8 | 36.0 |
| 2000  | 1     | 2.0      | 91.0 | **88.7** | 90.2 | 79.9 | 91.1 | 83.0 | **92.1** | 82.8 | 90.9 | 80.1 | 87.3 | 88.0 | 52.4 | 39.8 |
| 2500  | 1     | 2.0      | 93.4 | **91.2** | 92.9 | 81.0 | 93.6 | 84.0 | **94.3** | 82.8 | 93.4 | 81.1 | 90.1 | 90.8 | 57.9 | 42.4 |
| 1000  | 1000  | 0.75     | 2.0     | 68.4 | 67.6 | 62.0 | 67.1 | 66.3 | 68.1 | 67.7 | **70.8** | 64.7 | 70.3 | 65.8 | 63.6 | 28.7 | 34.8 |
| 2000  | 75.0  | 2.0     | **93.8** | 93.4 | 91.8 | 91.4 | 93.3 | 92.8 | **93.8** | 93.2 | 92.8 | 92.3 | 91.6 | 91.2 | 65.1 | 64.1 |
| 3000  | 0.75  | 2.0     | 97.8 | **97.4** | 97.0 | 95.2 | 97.6 | 96.4 | **97.9** | 96.3 | 97.4 | 95.6 | 96.6 | 96.5 | 78.8 | 74.1 |
| 4000  | 0.75  | 2.0     | **98.9** | 98.6 | 98.5 | 96.6 | 98.8 | 97.5 | **98.9** | 97.3 | 98.7 | 96.8 | 98.1 | 98.1 | 85.0 | 79.0 |
| 5000  | 0.75  | 2.0     | 99.3 | **99.1** | 99.0 | 97.3 | 99.3 | 98.1 | **99.4** | 97.9 | 99.2 | 97.5 | 98.7 | 98.8 | 88.3 | 81.9 |
| 5000  | 0.25  | 2.0     | 81.5 | 81.1 | 78.6 | 79.7 | 80.7 | 81.1 | 77.1 | 78.0 | 81.3 | 82.6 | 82.0 | 82.0 | 83.0 | **83.8** |
| 10000 | 0.50  | 1.5     | **80.3** | 79.9 | 72.7 | 71.9 | 77.8 | 77.1 | 77.1 | 76.1 | 75.5 | 74.7 | 73.8 | 73.8 | 57.8 | 57.3 |
| 15000 | 0.50  | 1.5     | **89.6** | **89.2** | 84.5 | 82.5 | 88.0 | 86.7 | 87.5 | 85.4 | 86.5 | 84.4 | 84.7 | 84.7 | 71.6 | 69.9 |
4 Discussion

Parameter choices in the simulation study. In the simulation study (Section 3) all data have been generated assuming that the disease prevalence is $k = 0.1$, the minor allele (disease allele) frequency is MAF = 0.1, and that the total population is in Hardy–Weinberg equilibrium (HWE). Further, we have only studied monotone genetic models with low to moderate effects size $\lambda^2$ and sample sizes in the order 500–5000 cases and 500–15000 controls. The conclusions to be drawn from the simulations study are thus only valid for these situations. However, some observations on the effect of changes to the set-up may be drawn.

The data are simulated in a case–control design. Keeping all other parameters fixed, the effect of doubling the disease prevalence is to double the probability of disease for each genotype. This will leave the genotype probabilities for the cases unchanged, and will only change the genotype probabilities for the controls slightly. We believe that the effect of changing the disease prevalence in our study will be minor.

There is a straightforward effect of changing the MAF. The simulation study used MAF = 0.1, and lowering the MAF will, most importantly, lead to a lower probability for the disease type homozygotes, $g_2 = \text{MAF}^2$. This will in turn lead to a greater imbalance in the expected cell counts for the six contingency table cells, influencing the validity of the asymptotic approximations in a negative manner.

Since we are working with test statistics that are based on genotype data, there is no need to assume HWE. We may generate data deviating from HWE by introducing an inbreeding coefficient when calculating genotype frequencies. In a data model with positive inbreeding coefficient the genotype frequencies for the two groups of homozygotes will increase and the genotype frequency for the heterozygote will decrease. We believe this will influence the disease homozygote group the most, and that this in turn will lead to a better balance in the expected cell counts for the six contingency table cells, thus, influencing the asymptotic approximations in a positive manner.

Validity and asymptotic methods. For candidate studies and intermediate follow-up studies producing a $p$-value for each genetic marker is in general of interest, while for GWA studies the main objective may be to provide a ranking of the genetic markers (with respect to increasing strength of the disease–genotype association).

We first discuss ranking of test statistics and ranking of $p$-value for GWA studies. Assume that in a case–control study with $n_1$ cases and $n_2$ controls we have studied $m$ genetic markers. These markers may in general come from a population with different genotype frequencies for each marker. The collected data would typically have different column margins.

For the test statistics CATT$_1/2$, Pearson, MIN2 and MERT, the asymptotic null distribution of the test statistics does not depend upon any unknown parameters, and also not on $n_1$ and $n_2$. This means that the rank of the $m$ genetic markers according to each of these test statistics will be the same as the rank according to the corresponding asymptotic $p$-value. For the test statistics MAX3, CMAX and CLRT the asymptotic null distribution is dependent on the estimated value of the genotype frequencies $g_0$, $g_1$, $g_2$. The ranking of the genetic markers by test statistic may differ from the ranking by the asymptotic $p$-values, but we believe that the difference in ranking is minor. For the ranking of the test statistics as compared to the ranking of the exact conditional enumeration $p$-value, the ranking of the exact conditional enumeration $p$-value will only be the same as the ranking of the test statistic for genetic markers with identical column margins. In a GWA study the ranking of the genetic markers based on a test statistic will be different from the ranking based on exact conditional enumeration $p$-values. To which extent the rankings differ may be a topic of further study.
When a \( p \)-value is calculated and used to guide the acceptance or rejection of one or many null hypotheses we would like to advocate using methods that produce valid \( p \)-values. Otherwise, violations of the single or multiple type I error will lead to loss of type I error control, and to optimistic power calculations.

The lack of validity of the \( p \)-values from asymptotic methods for low significance levels is in general not surprising, and has for other test statistics been observed by [Morris and Elston](2011).

In our simulation study the expected cell counts under the null hypothesis were \( g_i n_1 \) and \( g_i n_2 \) for genotypes \( i = 0, 1, 2 \) for cases and controls, respectively, which gave the smallest expected cell count of \( g_2 n_1 = 0.01 \cdot 500 = 5 \) over all our simulations. Thus, for all sample sizes studied there are no expected cell counts below 5 under the null hypothesis.

The asymptotic \( p \)-value calculated from the Pearson test statistic has been studied extensively. [Cochran (1954)] formulated the following rule of thumb for using the Pearson test statistic with the asymptotic chi-squared approximation for contingency tables with more than one degree of freedom (larger than \( 2 \times 2 \)). “If relatively few expectations are less than 5 (say in 1 cell out of 5 or more, or 2 cells out of 10 or more), a minimum expectation of 1 is allowable in computing \( \chi^2 \).” However, we found that the asymptotic \( p \)-value for the Pearson test did not keep its test size, especially for unbalanced sample sizes and low significance levels, even if all cells had expected count at least 5.

[Wise (1963)] found that errors in the chi-squared approximation in a multinomial situation are particularly small when the expected cell counts are equal or nearly so, and that these expected cell counts need not be large. Our results point towards a greater effect of equality in expected cells counts than of the numerical values of the expected cell counts. This can be seen by comparing the test sizes in Table 4. For \( n_1 = 500 \), observe that for the Pearson test statistics (and also for the MIN2, MAX3, CMAX and MERT) the violations in test size increase as \( n_2 \) increases from 500 to 2500. When \( n_2 \) increases, the expected cell counts for the controls will increase, but the difference between the expected cell counts between the cases and controls will also increase. The same pattern is seen for \( n_1 = 1000 \) as \( n_2 \) increases.

In addition to the Pearson test statistics, the CATT\(_{1/2}\) and the MERT test statistics follow the chi-squared and standard normal asymptotic null distributions. We believe that the observations on equality of expected cell counts for the Pearson test statistic will also apply to the other test statistics.

**Environmental covariates and logistic regression** All the methods considered in this presentation use only information on the disease phenotype and the genotype in order to calculate test statistics and \( p \)-values. However, data on environmental covariates may also have been collected. It is believed that complex diseases may be the result of an interplay between genetic markers and environmental covariates.

The asymptotic CATT\(_s\) test can be performed by first fitting a logistic regression to the disease status as response and the genotype as covariates, where the value 0 is used as coding for the wild type homozygotes, 1 for the disease homozygotes, and \( s \) for the heterozygotes, and then performing an asymptotic score test. This strategy is easily extended to include environmental covariates and interactions between environmental covariates and genotype. In a simulation study [Runde (2013)] found that the power gain in including an environmental covariate (when present) in a logistic regression score test is minimal compared to using the asymptotic CATT\(_s\) unless the effect of the (standardized) environmental covariate corresponds to at least an odds ratio of 5.

Robust methods for disease–genotype association with covariates are available. [So and Sham (2011)] has developed a MAX3 type method combining three asymptotic logistic regression
score test. However, the validity of the method, in particular when using low significance levels, has to our knowledge not been investigated. Exact conditional logistic regression are available (Mehta and Patel, 1995), but to our knowledge exact methods have not been investigated for robust methods with covariates.

5 Conclusions

We have studied seven test statistics that can be used to detect association between a dichotomous phenotype (disease) and genotype. We advocate that if you work with GWA studies, and would like to detect all monotone genetic models (including the recessive) with high power you should not use the CATT_{1/2} test statistics, but instead work with MAX3, CMAX or CLRT. If non-monotone effects are also of interest, the Pearson and MIN2 test statistics are found to perform well, also for over- and under dominant models (Joo et al., 2009).

We have shown how exact conditional enumeration is a valid and powerful competitor to simulated permutations and asymptotic approximation for producing p-values. Drawing simulated permutations is an inefficient way of calculating an exact conditional enumeration p-value for contingency tables of the size and order used in genetic association studies. In our simulation study calculating exact conditional enumeration p-values was done in a fraction of a second, even for the largest sample size considered in this presentation, (5000, 15000). Exact conditional enumeration should therefore be preferred to simulated permutations.

Further, it should be well known that p-values based on asymptotic approximations need not be valid, especially for small significance levels and unbalanced sample sizes, even when expected cell counts are at least 5. In an extensive simulation study we have seen that for the asymptotic approximation for the test statistics studied here, the violation of test size may be as large as 20 times the nominal level. Lastly, the fact that exact conditional enumeration methods give low power compared to asymptotic methods for small sample sizes for 2 × 2 contingency tables due to discreteness (Lehmann, 1993), is not transferrable to 2 × 3 contingency tables and the test statistics and sample sizes under study. In conclusion we find that exact conditional enumeration should also be preferred to asymptotic approximation, both with respect to test size, power and computational considerations.

Software

A C++ program using the GNU Scientific Library that takes the entries of a 2 × 3 table as input and gives the value of all seven test statistics together with the asymptotic and exact conditional enumeration p-values is available upon request.

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