Order-restricted Scores Test for the Evaluation of Population-based Case-control Studies when the Genetic Model is Unknown

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Abstract

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The Cochran-Armitage linear trend test for proportions is often used for genotype-based analysis of candidate gene association. Depending on the underlying genetic mode of inheritance, the use of model-specific scores maximises the power. Commonly, the underlying genetic model, i.e., additive, dominant or recessive mode of inheritance, is a priori unknown. Association studies are commonly analysed using permutation tests, where both inference and identification of the underlying mode of inheritance are important. Especially interesting are tests for case-control studies, defined by a maximum over a series of standardised Cochran-Armitage tests, because such a procedure has power under all three genetic models.

We reformulate the test problem and propose a conditional maximum test of scores-specific linear-by-linear association tests (Agresti 2002). For maximum-type, sum and quadratic test statistics the asymptotic expectation and covariance can be derived in a closed form and the limiting distribution is known. Both the limiting distribution and approximations of the exact conditional distribution can easily be computed using standard software packages. In addition to these technical advances, we extend the area of application to stratified designs, studies involving more than two groups and the simultaneous analysis of multiple loci by means of multiplicity-adjusted *p*-values for the underlying multiple Cochran-Armitage trend tests. The new test is applied to reanalyse a study investigating genetic components of different subtypes of psoriasis.

A new and flexible inference tool for association studies is available both theoretically as well as practically since already available software packages can be easily used to implement the suggested test procedures.

Keywords: genetic association, case-control study, robust trend test, maximum test, conditional inference.

1. Objectives

In population-based case-control studies the association between a candidate allele and a disease can be evaluated by the Cochran-Armitage (CA) trend test (Armitage 1955), regardless of whether or not Hardy-Weinberg equilibrium holds (Sasieni 1997). The CA test is based on a set of scores assigned to the alleles. For genotypes *aa*, *Aa*, or *AA*, with *A* denoting a high risk

Table 1: Genotype distributions for cases and controls.

	Cases	Controls	Total
aa	r_{aa}	s_{aa}	n_{aa}
aA	r_{aA}	s_{aA}	n_{aA}
AA	r_{AA}	s_{AA}	n_{AA}
Total	R	S	N

candidate allele and a any of the other alleles, three-dimensional score vectors optimising the power of the CA test against dominant, additive, and recessive alternatives can be defined. If the underlying mode of inheritance is known, the choice of an appropriate score vector for the trend test is obvious. However, in situations where the underlying genetic model is unknown choosing the *wrong* score vector leads to a substantial loss of power as shown by Freidlin, Zheng, Li, and Gastwirth (2002). Therefore, inference procedures with good power under *all* three genetic models are of special interest. An appealing approach is to construct a test based on all three possible trend tests, for example utilising the maximum of the standardised test statistics of the CA tests which are optimal under the dominant, additive, and recessive model. An unconditional version of this test was proposed and investigated by Freidlin *et al.* (2002). The distribution of this maximum test, called MAX test hereafter, under the null hypothesis of equal genotype distribution in cases and controls is approximated by simulation procedures by Freidlin *et al.* (2002) since the unconditional asymptotic distribution is hard to derive.

In this paper, we show that the conditional MAX test is a simple special case of a general class of linear statistics whose conditional distribution is easy to compute. Thus, without additional theoretical effort, the MAX test enjoys all the nice properties of this class of conditional tests. Therefore, we embed the MAX test as suggested by Freidlin *et al.* (2002) into the flexible framework for conditional independence tests introduced by Strasser and Weber (1999). The merits of doing so are i) the conditional reference distribution for the MAX test can be easily approximated either by evaluating a three-dimensional normal distribution or by conditional Monte Carlo experiments, ii) tests for stratified designs, designs with more than two groups and two or more loci can be defined in a rather straightforward way, iii) the most likely underlying mode of inheritance can be estimated by multiplicity-adjusted p-values for the three CA statistics under consideration, iv) the simultaneous analysis of multiple loci, and v) the analysis of genetic association studies using the MAX test and its newly introduced extension can be performed by already available software implementations of the Strasser and Weber (1999) framework.

2. Maximum Test

For case-control studies investigating the association between a binary phenotype and the measured alleles of a candidate gene the data are typically presented as empirical genotype distribution in each group. For a simple bi-allelic marker the data can be presented in a 3×2 contingency table, where A is the high risk candidate allele and a is any of the other alleles (see Table 1).

Table 2: Genotype distribution reformulated.

i	$\mathbf{Y}_i$	$\mathbf{X}_i$	w_i	$h(\mathbf{Y}_i)$	$g_{\mathrm{add}}(\mathbf{X}_i)$	$g_{\mathrm{dom}}(\mathbf{X}_i)$	$g_{\mathrm{rec}}(\mathbf{X}_i)$
1	Case	aa	r_{aa}	1	0	0	0
2	Case	Aa	r_{aA}	1	1	1	0
3	Case	AA	r_{AA}	1	2	1	1
4	Control	aa	s_{aa}	0	0	0	0
5	Control	Aa	s_{aA}	0	1	1	0
6	Control	AA	s_{AA}	0	2	1	1

We are interested in a comparison of the genotype distributions, i.e., the penetrances $f_j = P(\text{case}|j)$ for $j \in \{aa, aA, AA\}$:

$$H_0 : f_{aa} = f_{aA} = f_{AA} \text{ vs. } H_1 : f_{aa} \leq f_{aA} \leq f_{AA}$$

where at least one of the inequalities in the alternative is strict.

The CA test statistic with scores $\xi = (\xi_{aa}, \xi_{aA}, \xi_{AA})$ is essentially (modulo standardisation)

$$\mathrm{CA}(\xi) = \sum_{j \in \{aa, aA, AA\}} \xi_j r_j. \tag{1}$$

If the mode of inheritance is best described by the dominant model, the scores $\xi_{\mathrm{dom}} = (0, 1, 1)$ ($f_{aa} < f_{aA} = f_{AA}$) will lead to a trend test with maximal power. Under the recessive model the score vector $\xi_{\mathrm{rec}} = (0, 0, 1)$ ($f_{aa} = f_{aA} < f_{AA}$) is power optimal whereas a linear trend represented by scores $\xi_{\mathrm{add}} = (0, 1, 2)$ should be chosen when the mode if inheritance is additive, i.e., $f_{aa} < f_{aA} < f_{AA}$ (Sasieni 1997; Slager and Schaid 2001). However, the underlying genetic model is rarely known *a priori*. Motivated by the problem of choosing the 'right' score vector, Freidlin *et al.* (2002) proposed the MAX unconditional test as the maximum of three standardised CA tests with scores $\xi_{\mathrm{dom}}, \xi_{\mathrm{add}}$, and ξ_{rec} as a global test for association. A similar approach (Zheng, Freidlin, Li, and Gastwirth 2003; Zheng 2008) is to introduce a parameter η for the score vector $\xi_\eta = (0, \eta, 1)$ and to choose η in a data-driven way. This procedure (based on a grid of η values) is a special case of the general framework described in the following, see Appendix A for more details.

In the following, we reformulate the test problem and embed the MAX test into a general framework for conditional inference procedures, derive its limiting distribution and propose extensions to stratified designs, more than two groups and multiple loci.

2.1. Reformulation of the Problem

Let $\mathbf{Y}_i$ denote the case and control status and $\mathbf{X}_i$ the genotype for all cells $i = 1, \ldots, n = 6$. The weights w_i represent the number of observations in each cell with total number of observations $N = \sum_i w_i$. The influence function h provides us with a zero-one dummy coding of the groups (being one for cases and zero for controls). Moreover, three transformations g of the genotype are under test: g_{dom} assigns scores ξ_{dom} to genotypes (aa, Aa, AA), g_{add} assigns scores ξ_{add} and g_{rec} implements scores ξ_{rec}, cf. Table 2.

2.2. Inference Problem and Linear Statistic

We are interested in testing the null hypothesis of independence of grouping $\mathbf{Y}$ and genotype $\mathbf{X}$, i.e., the equality of the distribution $D(\mathbf{Y})$ to the conditional distribution $D(\mathbf{Y}|\mathbf{X})$ of $\mathbf{Y}$ given $\mathbf{X}$

$$H_0 : D(\mathbf{Y}|\mathbf{X}) = D(\mathbf{Y})$$

against ordered alternatives. First, we define a three-dimensional statistic $\mathbf{T}$, each dimension being associated with one of the scores g_{add}, g_{dom}, and g_{rec}. Each statistic is defined by the sum of the scores multiplied by the weights associated with cases, i.e., is equivalent to the Cochran-Armitage statistic (1):

$$\mathbf{T} = (\mathbf{T}_{\mathrm{add}}, \mathbf{T}_{\mathrm{dom}}, \mathbf{T}_{\mathrm{rec}}) = \sum_{i=1}^{n} w_i g(\mathbf{X}_i) h(\mathbf{Y}_i) \in \mathbb{R}^3 \tag{2}$$

with $g(\mathbf{X}_i) = (g_{\mathrm{add}}(\mathbf{X}_i), g_{\mathrm{dom}}(\mathbf{X}_i), g_{\mathrm{rec}}(\mathbf{X}_i))$. Thus, the three-dimensional linear statistic $\mathbf{T}$ is the vector of the unstandardised Cochran-Armitage statistics $(\mathrm{CA}(\xi_{\mathrm{dom}}), \mathrm{CA}(\xi_{\mathrm{add}}), \mathrm{CA}(\xi_{\mathrm{rec}}))$ for the dominant, additive, and recessive model.

2.3. Conditional Expectation and Covariance

The distribution of $\mathbf{T}$ depends on the joint distribution of $\mathbf{Y}$ and $\mathbf{X}$, which is unknown under almost all practical circumstances. At least under the null hypothesis one can dispose of this dependency by fixing the genotypes and conditioning on all possible permutations of the groups. This principle leads to test procedures known as *permutation tests*. Strasser and Weber (1999) derived closed-form expressions for the conditional expectation $\mu \in \mathbb{R}^{pq}$ and covariance $\Sigma \in \mathbb{R}^{3 \times 3}$ of $\mathbf{T}$ under H_0 given all permutations of the groupings.

The conditional expectation of the influence function h is

$$\mathsf{E}(h) = N^{-1} \sum_i w_i h(\mathbf{Y}_i) \in \mathbb{R}$$

with corresponding variance

$$\mathbb{V}(h) = N^{-1} \sum_i w_i \left(h(\mathbf{Y}_i) - \mathsf{E}(h) \right)^2 .$$

The conditional expectation of the linear statistic $\mathbf{T}$ is

$$
\begin{aligned}
\mu &= \mathsf{E}(\mathbf{T}) = \mathsf{E}(h) \sum_{i=1}^{n} w_i g(\mathbf{X}_i), \\
\Sigma &= \mathbb{V}(\mathbf{T}) \\
&= \frac{N}{N-1} \mathbb{V}(h) \times \left(\sum_i w_i \left(g(\mathbf{X}_i) g(\mathbf{X}_i)^\top \right) \right) \\
&\quad - \frac{1}{N-1} \mathbb{V}(h) \times \left(\sum_i w_i g(\mathbf{X}_i) \right) \left(\sum_i w_i g(\mathbf{X}_i) \right)^\top .
\end{aligned}
\tag{3}
$$

The three-dimensional expectation μ and the three diagonal elements of the covariance matrix Σ contain the mean and the variances for the additive, dominant and recessive (unstandardised) Cochran-Armitage statistics under H_0, as given in (1) and (2), respectively.

Note that the complete covariance structure, and thus the correlation between the elements of the three-dimensional statistic $\mathbf{T}$, is known and can be computed for the data at hand. The corresponding correlation matrix coincides with the correlations obtained for the three CA test statistics by Freidlin *et al.* (2002).

2.4. Test Statistics

Based on the three-dimensional statistic $\mathbf{T}$ and its expectation μ and covariance matrix Σ, we can easily construct test statistics and derive their distribution under the conditions described in the null hypothesis. As the number of observations N tends to infinity, Strasser and Weber (1999) proved that the limiting distribution of the three-dimensional statistic $\mathbf{T}$ is a three-dimensional normal distribution with expectation μ and covariance Σ. Thus, the asymptotic distribution of a maximum-type statistic

$$c_{\max}(\mathbf{T}, \mu, \Sigma) = \max \left| \frac{\mathbf{T} - \mu}{\mathrm{diag}(\Sigma)^{1/2}} \right|$$

can be evaluated by computing three-dimensional normal probabilities. Here, $\mathrm{diag}(\Sigma)^{1/2}$ are the conditional standard deviations of the elements of $\mathbf{T}$. Alternatively, either the sum (or average) statistic

$$c_{\mathrm{sum}} = \frac{\mathbf{1}^\top \mathbf{T} - \mathbf{1}^\top \mu}{\mathbf{1}^\top \Sigma \mathbf{1}}, \quad \mathbf{1} = (1, 1, 1)^\top$$

or a quadratic form based on the Moore-Penrose inverse Σ^+ of the conditional covariance matrix Σ, i.e.,

$$c_{\mathrm{quad}}(\mathbf{T}, \mu, \Sigma) = (\mathbf{T} - \mu)^\top \Sigma^+ (\mathbf{T} - \mu)$$

can be used and follow a standard normal or a χ^2 distribution with two degrees of freedom, respectively. The sum and quadratic form statistics, which are competitors for the MAX test statistic, reveal high power for an average alternative while the maximum-type form for a particular genetic alternative. In addition, simple linear combinations of the standardised optimum tests for each of the models have been proposed (Gastwirth 1985). Often the most appropriate test is not the average of all these tests but a linear combination of a few "extreme" members. Nonlinear MAX type tests have greater efficiency robustness properties when the minimum correlation between the optimal tests for the models considered is less than .70 (Freidlin, Podgor, and Gastwirth 1999), therefore, we focus on the MAX test. A further advantage of maximum-type statistics is that information about the likely genetic model underlying the data is available using multiplicity-adjusted p-values. Note that, under any circumstances, the exact conditional distribution can be approximated by conditional Monte Carlo methods, which is especially attractive for small sample sizes N when we can not expect asymptotics to work well.

2.5. Illustration

In order to compare the conditional test and its implementation with the unconditional results reported by Freidlin *et al.* (2002), we reanalyse a study on the association between a variant of the epidermal growth factor (EGF) gene and malignant melanoma according to

Table 3: Melanoma data.

	In situ	Control	Total
AA	6	32	38
AG	8	47	55
GG	10	20	30
Total	24	99	123

Table 4: MAX test for Melanoma data with linear statistic $\mathbf{T}$, its conditional expectation μ, standard deviation σ and corresponding standardised statistic along with multiplicity-adjusted p-values.

	$\mathbf{T}$	μ	σ	$(\mathbf{T}-\mu)/\sigma$	p_{asympt}	$p_{\text{step-down}}$
dominant	18	16.5854	2.0391	0.6938	0.3907	0.3302
additive	28	22.4390	3.2687	1.7013	0.0868	0.0654
recessive	10	5.8537	1.8951	2.1879	0.0303	0.0359

Shahbazi, Pravica, Nasreen, Fakhoury, Fryer, Strange, Hutchinson, Osborne, Lear, Smith, and Hutchinson (Table 3, 2002).

The linear statistic $\mathbf{T}$, its conditional expectation μ, the standard deviations $\sigma = \sqrt{\text{diag}(\Sigma)}$, and the corresponding standardised CA statistics are given in Table 4. In addition, we immediately are provided with the covariance matrix

$$\Sigma = \begin{pmatrix} 4.1579 & 5.6255 & 1.4675 \\ 5.6255 & 10.6845 & 5.0590 \\ 1.4675 & 5.0590 & 3.5915 \end{pmatrix}$$

and corresponding correlation matrix

$$\text{cor}(\Sigma) = \begin{pmatrix} 1.0000 & 0.8440 & 0.3798 \\ 0.8440 & 1.0000 & 0.8167 \\ 0.3798 & 0.8167 & 1.0000 \end{pmatrix}$$

These values are similar to the correlations between the three different CA test statistics as reported by Freidlin *et al.* (2002).

The MAX test has a test statistic equal to 2.1879 and its asymptotic p-value is 0.0303 (the minimum of p_{asympt} in Table 4) which is roughly the same p-value as shown in Table 8 of Freidlin *et al.* (2002). However, this global p-value does not give any information about the underlying genetic model. Multiplicity-adjusted p-values (p_{asympt} in Table 4) for each of the dominant, additive, and recessive tests indicate which mode of inheritance describes the data best (see Section 4 in addition): These results suggest that the cancer follows a recessive model, although an additive or intermediate model cannot be ruled out based on these data.

We might want to check whether the asymptotic approximation work well enough in this situation. The exact conditional p-value is approximated by a conditional Monte Carlo procedure

with 49999 random permutations of the data and the corresponding step-down multiplicity-adjusted p-values (Westfall and Young 1993) are given as $p_{\text{step-down}}$ in Table 4. The small differences between the asymptotic and approximated p-values indicates that using the asymptotic distribution is adequate.

2.6. Generalisations

A straightforward generalisation is the consideration of $3 \times k$ tables instead of 3×2 tables, where sub-types of cases are compared with a control. For example, the genotype distribution of the control can compare the genotype of cases with early and late onset of a certain disease. A score can be attached to each group, for example 1 to the control group and $-1/2$ for both the early and late onset cases leading to a linear-by-linear association test. Alternatively, a trend in the onset of the disease can be described by scores $0, 1, 2$ for the three groups.

In stratified designs, only permutations within each stratum, gender or family history, are admissible; therefore, the expectation μ and covariance Σ has to be computed separately for each stratum and is then aggregated over all possible strata. A special version of a stratified design is the commonly used meta-analysis, including several independent studies as strata, see e.g. Kavvoura and Ioannidis (2008). In the absence of a a priori assumption for a particular mode of inheritance, recently Salanti and Higgins (2008) proposed a Bayesian approach.

In a recent meta-analysis of genome-wide association studies variants on chromosome 9p21.3 were identified affecting the coronary artery disease (Schunkert, Gotz, Braund, McGinnis, Tregouet, Mangino, Linsel-Nitschke, Cambien, Hengstenberg, Stark, Blankenberg, Tiret, Ducimetiere, Keniry, Ghori, Schreiber, El Mokhtari, Hall, Dixon, Goodall, Liptau, Pollard, Schwarz, Hothorn, Wichmann, Konig, Fischer, Meisinger, Ouwehand, Deloukas, Thompson, Erdmann, Ziegler, and Samani 2008) where all seven studies revealed the same additive mode of inheritance using the here proposed approach.

Finally, it is interesting to consider multiple loci, i.e., multiple genotype distributions, simultaneously. For two loci, we can look at all six CA tests by defining a linear statistic $\mathbf{T}$ containing the three CA tests for the first as well as the three CA tests for the second locus. As a consequence, we can compute the complete covariance matrix and take the underlying correlations between the two loci as well as between the three genetic models into account.

3. Illustration and Application

Reich, Mössner, König, Westphal, Ziegler, and Neumann (2002) investigate the association between psoriasis and polymorphisms of genes encoding tumour necrosis Factor-α and Interleukin-1β where for the IL1B_511 locus the related 3×2 table data are given in Table 5. A control group and two groups of affected people with early and late onset of the disease are under test. One is interested in detecting any deviation from independence of genotype distribution for both loci and the three groups in either females and / or males. Attaching scores $1, -1/2, -1/2$ to the control, early and late onset group results in a linear statistic $\mathbf{T}$ with six elements: three models for each of the two loci.

The multiplicity-adjusted p-values in Table 6 indicate that there is a strong deviation from independence for the TNFA_238 locus. The recessive model has the largest p-value and thus it is not likely that this model is true. The p-values for the dominant and the additive are extremely small, so either of these models could have generated the data. We can simulta-

Table 5: Psoriasis data

IL1B_511 locus

Male		Control	Early Onset	Late Onset	Total
	CC	75	54	29	158
	CT	93	44	13	150
	TT	14	7	4	25
	Total	182	105	46	333
Female		Control	Early Onset	Late Onset	Total
	CC	76	26	17	119
	CT	69	20	10	99
	TT	18	5	2	25
	Total	163	51	29	243

TNFA_238 locus

Male		Control	Early Onset	Late Onset	Total
	GG	170	71	40	281
	GA	12	33	6	51
	AA	0	1	0	1
	Total	182	105	46	333
Female		Control	Early Onset	Late Onset	Total
	GG	146	43	24	213
	GA	17	8	5	30
	AA	0	0	0	0
	Total	163	51	29	243

neously reject the null hypothesis of independence between the genotype distribution of the IL1B_511 locus and the three groups. Here, the dominant model seems to explain the data best.

Our analysis improves upon the original analysis of these data by Reich *et al.* (2002) with respect to three points: All three groups and the stratification by gender are taken into account and and the new test makes use of the correlation between the two loci instead of applying a Bonferroni correction in order to maintain an overall significance level.

Recently, Bagos and Nikolopoulos (2007) proposed a penalty-free selection approach for the underlying mode of inheritance based on the parameter estimates in a logistic regression model. Here, we re-analyse their meta-analysis data on the association of KIR6.2 gene polymorphism with type II diabetes (Table 1 on page 3 in Bagos and Nikolopoulos 2007).

4. Simulation Experiments

It might be questioned if the minimal p-value can be observed for the correct mode of in-

Table 6: MAX test for psoriasis data: Asymptotic multiplicity-adjusted p-values.

	TNFA_238	IL1B_511
dominant	< 0.0001	0.0407
additive	< 0.0001	0.1051
recessive	0.7241	0.9819

Table 7: MAX test for Type II diabetes data with linear statistic $\mathbf{T}$, its conditional expectation μ, standard deviation σ and corresponding standardised statistic along with multiplicity-adjusted p-values.

	$\mathbf{T}$	μ	σ	$(\mathbf{T} - \mu)/\sigma$	p_{asympt}	$p_{\mathrm{step\text{-}down}}$
dominant	337	320.4287	7.0531	2.3495	0.0207	0.0112
additive	438	404.0265	10.1618	3.3433	0.0012	0.001
recessive	101	83.5979	5.2912	3.2889	0.0012	0.0013

heritance with high probability and thus how good the 'estimator' is under practical circumstances. The frequency of correct model identifications and the power of the MAX test is investigated in some simple situations in the following.

Many different patterns of penetrances f_j, $j \in \{aa, aA, AA\}$, disease prevalence p, sample size of cases and controls R, S can be investigated in a simulation study. We will focus on a high prevalent disease (i.e., $p = 0.5$), penetrances according to an additive, recessive and dominant genetic model (as well as no association characterising the null hypothesis) for a total sample size of $N = 400$ divided into the balanced $R = S = 200$ and several unbalanced sampling schemes. Unbalanced data are of interest because real data examples exist with seriously higher control sample size, see e.g. the data in Table 3, or with more cases, see e.g. the IL13 polymorphism in atopic dermatitis Neuhäuser (2002), Table 4. For the proposed MAX test both the global power π_{global}(the decision rate in favour of any alternative) and the correct model identification rates $\psi_{\mathrm{add}}, \psi_{\mathrm{rec}}, \psi_{\mathrm{dom}}$ are compared with the power of the individual genetic model tests π_{add}, π_{rec}, π_{dom} in Table 8.

Per definition all tests control the type I error rates. Clearly, the power is maximal for the individual, unadjusted tests when the genetic model is known (bold marked). But the a priori knowledge of the genetic model is commonly unrealistic. For balanced samples sizes the power of the MAX test is independent of the underlying genetic model and marginal smaller compared with the maximum power for the known model. Additionally to the global decision that a significant association exists, the MAX test provide an adjusted p-value for the most likely genetic model. In this case, the identification of the additive model is most difficult because of the two equal competitors; whereas the identification of the dominant or recessive model is easier because the additive model is the only competitor. For unbalanced designs the power decreases although the total sample size remains constant.

Table 8: Type I error rate and empirical power estimates (π) for prevalence $p = 0.5$ based on 10000 runs with sample sizes R (cases) and S (controls) along with correct model identification rates ψ.

Model	R	S	π_{global}	ψ_{add}	ψ_{rec}	ψ_{dom}	π_{add}	π_{rec}	π_{dom}
Null	200	200	0.048	0.012	0.017	0.019	0.051	0.047	0.049
dominant	200	200	0.85	0.13	0.01	*0.71*	0.75	0.23	**0.91**
additive	200	200	0.84	*0.53*	0.10	0.21	**0.88**	0.71	0.78
recessive	200	200	0.86	0.16	*0.69*	0.01	0.80	**0.91**	0.28
dominant	100	300	0.72	0.16	0.01	*0.55*	0.63	0.21	**0.80**
additive	100	300	0.73	*0.43*	0.14	0.16	**0.78**	0.60	0.65
recessive	100	300	0.77	0.16	*0.60*	0.01	0.67	**0.82**	0.22
dominant	300	100	0.76	0.11	0.01	*0.64*	0.66	0.19	**0.82**
additive	300	100	0.75	*0.42*	0.09	0.24	**0.79**	0.59	0.69
recessive	300	100	0.75	0.17	*0.55*	0.01	0.69	**0.82**	0.24

5. Computational Details

The **coin** add-on package (Hothorn, Hornik, van de Wiel, and Zeileis 2006, 2008, 2009) to the R system for statistical computing (R Development Core Team 2009) provides an implementation of the conditional inference framework sketched in this section. The analysis of an association study by the MAX test only requires the user to set-up the score function g. Then, the function `independence_test` can be used to perform the MAX test and to compute multiplicity-adjusted p-values.

6. Conclusions

We propose a flexible approach to permutation tests for association of a bi-allelic marker with a disease in population-based case-control studies. The joint conditional asymptotic distribution of the three underlying linear association tests, i.e., Cochran-Armitage tests with optimal scores for additive, dominant, and recessive modes of inheritance, is known and can be used to approximate the distribution of the corresponding maximum statistic. Not only a global p-value can be derived this way but also multiplicity-adjusted p-values for each of the individual models. When the mode of inheritance is unknown, remarkably high correct model selection rates can be achieved. Based on a general framework for conditional inference we extend the MAX test to stratified designs, $3 \times k$ tables as well as multiple loci. Correlations between loci and corresponding association tests are taken into account leading to more powerful multiple test procedure. Further modifications and extensions and specific choices of g and h are described by Hothorn *et al.* (2006) and Hothorn *et al.* (2008). For small sample sizes, a better approximation of the p-values can be obtained from Monte Carlo resampling techniques. For genome-wide association studies, computing time is a limiting factor; a further advantage for the above approach (Ziegler, Koenig, and Thompson 2008).

The proposed procedures are easily applicable using the computational tools provided by the R add-on package **coin** as illustrated in Appendix A. A future modification will be the use of

model-specific genomic-control corrected tests analogously to Zang, Zhang, Yang, and Zheng (2007) in the possible case of population stratification.

7. Acknowledgements

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A. Example Analyses

The Melanoma data are represented by a `table` object in R as follows:

```
R> me <- as.table(matrix(c( 6,  8, 10,
                           32, 47, 20), byrow = TRUE, nrow = 2,
       dimnames = list(Group = c("In situ", "Control"),
                        Genotype = c("AA", "AG", "GG"))))
R> me <- t(me)
R> me
```

```
         Group
Genotype In situ Control
      AA       6      32
      AG       8      47
      GG      10      20
```

The function *g* is implemented by the following function:

```
R> add <- c(0, 1, 2)
R> dom <- c(0, 1, 1)
R> rec <- c(0, 0, 1)
R> g <- function(x) {
       x <- unlist(x)
       cbind(dominant = dom[x], additive = add[x], recessive = rec[x])
   }
```

which then sets up the MAX test for the Melanoma data:

```
R> library("coin")
R> it <- independence_test(me, xtrafo = g, alternative = "greater")
R> it
```

```
        Asymptotic General Independence Test

data:  Group by Genotype (AA, AG, GG)
maxT = 2.1879, p-value = 0.03042
alternative hypothesis: greater
```

The multiplicity-adjusted *p*-values for both inference and estimating the underlying mode of inheritance are computed via:

```
R> pvalue(it, method = "single-step")
```

```
  dominant   additive  recessive
0.39063737 0.08676118 0.03040045
```

The score independent approach by Zheng (2008) maximises the Cochran-Armitage statistic over potential score vectors $\xi_\eta = (0, \eta, 1)$, i.e., one is interested in the test statistic $c_{\max}$ where the maximium is taken over a grid of η values. This procedure can be performed by choosing appropriate transformation g, i.e., a grid of η values in $[0, 1]$

```
R> gZheng <- function(x) {
       x <- unlist(x)
       eta <- seq(from = 0, to = 1, by = 0.01)
       tr <- sapply(eta, function(n) c(0, n, 1)[x])
       colnames(tr) <- paste("eta", eta, sep = "_")
       tr
   }
R> itZ <- independence_test(me, xtrafo = gZheng, alternative = "greater")
R> itZ


        Asymptotic General Independence Test

data:  Group by Genotype (AA, AG, GG)
maxT = 2.1879, p-value = 0.03151
alternative hypothesis: greater


R> pvalue(itZ, method = "single-step")


     eta_0    eta_0.01   eta_0.02   eta_0.03   eta_0.04
0.03151304 0.03162379 0.03176212 0.03191972 0.03209197
  eta_0.05   eta_0.06   eta_0.07   eta_0.08   eta_0.09
0.03228663 0.03250561 0.03274823 0.03301281 0.03330016
   eta_0.1   eta_0.11   eta_0.12   eta_0.13   eta_0.14
0.03361386 0.03395610 0.03432383 0.03472546 0.03515723
  eta_0.15   eta_0.16   eta_0.17   eta_0.18   eta_0.19
0.03561464 0.03610973 0.03663956 0.03720648 0.03781138
   eta_0.2   eta_0.21   eta_0.22   eta_0.23   eta_0.24
0.03845817 0.03914792 0.03987640 0.04065208 0.04147678
  eta_0.25   eta_0.26   eta_0.27   eta_0.28   eta_0.29
0.04235435 0.04328269 0.04426266 0.04530451 0.04640300
   eta_0.3   eta_0.31   eta_0.32   eta_0.33   eta_0.34
0.04756671 0.04879473 0.05009090 0.05145841 0.05289975
  eta_0.35   eta_0.36   eta_0.37   eta_0.38   eta_0.39
0.05441897 0.05601775 0.05770110 0.05946786 0.06132908
   eta_0.4   eta_0.41   eta_0.42   eta_0.43   eta_0.44
0.06327947 0.06533041 0.06747833 0.06973053 0.07209088
  eta_0.45   eta_0.46   eta_0.47   eta_0.48   eta_0.49
0.07456038 0.07714360 0.07984304 0.08266065 0.08560310
   eta_0.5   eta_0.51   eta_0.52   eta_0.53   eta_0.54
0.08867050 0.09186639 0.09519253 0.09865287 0.10224856
  eta_0.55   eta_0.56   eta_0.57   eta_0.58   eta_0.59
0.10598309 0.10985423 0.11386954 0.11802337 0.12232122
```

```
   eta_0.6    eta_0.61    eta_0.62    eta_0.63    eta_0.64
0.12676362 0.13134787 0.13608048 0.14095192 0.14596886
   eta_0.65    eta_0.66    eta_0.67    eta_0.68    eta_0.69
0.15112707 0.15642563 0.16186213 0.16743632 0.17314356
    eta_0.7    eta_0.71    eta_0.72    eta_0.73    eta_0.74
0.17898205 0.18494849 0.19104191 0.19725298 0.20358348
   eta_0.75    eta_0.76    eta_0.77    eta_0.78    eta_0.79
0.21002529 0.21657605 0.22322887 0.22998050 0.23682421
    eta_0.8    eta_0.81    eta_0.82    eta_0.83    eta_0.84
0.24375584 0.25076897 0.25785709 0.26501653 0.27223996
   eta_0.85    eta_0.86    eta_0.87    eta_0.88    eta_0.89
0.27952144 0.28685492 0.29423443 0.30165464 0.30910862
    eta_0.9    eta_0.91    eta_0.92    eta_0.93    eta_0.94
0.31659023 0.32409437 0.33161541 0.33914685 0.34668311
   eta_0.95    eta_0.96    eta_0.97    eta_0.98    eta_0.99
0.35421895 0.36174935 0.36926863 0.37677208 0.38425498
      eta_1
0.39171216
```

The test statistic takes its maximum for the score $(0, 0, 1)$ (as above), its corresponding adjusted p-value is only marginally larger compared to the MAX test based on three score vectors (0.03151 vs. 0.03042).

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