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Two-Locus Disease Models with Two Marker Loci: The Power of Affected-Sib-Pair Tests

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Summary

Recently, Schork et al. found that two-trait-locus, two-marker-locus (parametric) linkage analysis can provide substantially more linkage information than can standard one-trait-locus, one-marker-locus methods. However, because of the increased burden of computation, Schork et al. do not expect that their approach will be applied in an initial genome scan. Further, the specification of a suitable two-locus segregation model can be crucial. Affected-sib-pair tests are computationally simple and do not require an explicit specification of the disease model. In the past, however, these tests mainly have been applied to data with a single marker locus. Here, we consider sib-pair tests that make it possible to analyze simultaneously two marker loci. The power of these tests is investigated for different (epistatic and heterogeneous) two-trait-locus models, each trait locus being linked to one of the marker loci. We compare these tests both with the test that is optimal for a certain model and with the strategy that analyzes each marker locus separately. The results indicate that a straightforward extension of the well-known mean test for two marker loci can be much more powerful than single-marker-locus analysis and that its power is only slightly inferior to the power of the optimal test.

Introduction

The availability of increasingly denser maps of polymorphic markers makes it straightforward to locate simple Mendelian diseases. However, such complex genetic diseases as psychiatric disorders or asthma are by definition not controlled by only a single disease locus, but can be expected to involve multiple genetic and/or environmental factors. For the purpose of linkage analysis, it is common practice to model complex diseases such as these as single-locus diseases with reduced penetrance. The appropriateness of this approximation has been considered by several investigators (Durner et al. 1992; Goldin 1992; Vieland et al. 1992, 1993). The general conclusion of these papers is that using a single-locus disease model is only slightly less powerful than analyzing the data under the correct model. However, they all assumed that there is only a single genetic marker available. In contrast, Schork et al. (1993) found that the simultaneous inclusion of two marker loci, each of them linked to one of the two disease loci, can substantially increase the power to detect linkage.

As noted by Schork et al. (1993), the cost for the increased linkage information obtained by the two-trait, two-marker approach is an increased burden of computation. Therefore, these authors do not expect that their method will be applied in an initial genome scan. Further, for their linkage analysis, Schork et al. (1993) assumed that the parameters of the model (penetrances and gene frequencies at the two disease loci) are known; that is, they carried out the calculation of lod scores under the generating model for their data. Even for single-locus models, the specification of appropriate parameter values is a well-known problem (Ott 1991). For two-locus disease models, penetrance values for nine different disease genotypes have to be specified. In most situations, assuming these penetrances to be known will not be realistic.

Affected-sib-pair methods do not require the specification of the mode of inheritance. Because of this and because of their computational simplicity, these methods have been widely used as a tool especially suited to screen a large quantity of marker data. The method is based on a sample of nuclear families with two affected children. The empirical distribution of the number of marker alleles shared identical by descent (IBD) by the two affected sibs is compared with its theoretical distribution in case of no linkage. A variety of statistical tests have been proposed for this situation; more recent examples have been given by Schaid and Nick (1990), Faraway (1993), and Holmans (1993). So far, these papers have been concerned with a single marker locus only. To our knowledge, only Dizier and Clerget-Darpoux (1986) considered the joint distribution of identity for HLA and Gm in a sample of diabatic sibs. However, they used a 0-1 variable of phenotypic identity, instead of IBD, for Gm.

The present paper addresses the following two ques-

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Table 1

Description of Two-Locus Segregation Models

Model	Two-Locus Penetrances[a]									Parameter Values			Population-Risk Characteristics[b]		
	f_{22}	f_{21}	f_{20}	f_{12}	f_{11}	f_{10}	f_{02}	f_{01}	f_{00}	p_1	p_2	$\phi = \phi_1 = \phi_2$	K_p	K_{po}	K_{sib}
Ep-1	ϕ	ϕ	0	ϕ	ϕ	0	0	0	0	.210	.210	.707	.100	.300	.317
Ep-2	ϕ	ϕ	0	0	0	0	0	0	0	.600	.199	.778	.100	.300	.329
Ep-3	ϕ	0	0	0	0	0	0	0	0	.577	.577	.900	.100	.300	.348
Ep-4	ϕ	ϕ	0	ϕ	0	0	ϕ	0	0	.372	.243	.911	.100	.300	.370
Ep-5	ϕ	ϕ	0	ϕ	0	0	0	0	0	.349	.349	.799	.100	.300	.328
Ep-6	0	ϕ	ϕ	ϕ	0	0	ϕ	0	0	.190	.190	1.000	.070	.209	.361
Het-1	φ	φ	ϕ_1	φ	φ	ϕ_1	ϕ_2	ϕ_2	0	.053	.053	.495	.100	.300	.303
Het-2	φ	φ	ϕ_1	ϕ_2	ϕ_2	0	ϕ_2	ϕ_2	0	.279	.040	.660	.100	.301	.342
Het-3	φ	ϕ_1	ϕ_1	ϕ_2	0	0	ϕ_2	0	0	.194	.194	1.000	.074	.222	.377
S-1	ϕ	ϕ	ϕ	ϕ	ϕ	ϕ	ϕ	ϕ	0	.052	.052	.522	.100	.300	.303
S-2	1	1	1	ϕ	ϕ	0	ϕ	ϕ	0	.228	.045	.574	.100	.300	.372
S-3	1	1	ϕ	1	ϕ	0	ϕ	0	0	.194	.194	.512	.100	.300	.317

[a] $\varphi = \phi_1 + \phi_2 - \phi_1 \cdot \phi_2$.

[b] Prevalence (K_p) and recurrence risk for offspring (K_{po}) and sib (K_{sib}).

tions: (1) is there any gain over successive testing for linkage at each marker locus in considering the joint distribution of IBD scores for two-marker systems? and (2) which test should be employed? To answer these questions, we considered a wide range of two-trait-locus heterogeneity and epistatic models. For each of these disease models, we then evaluated the behavior of different single-marker and two-marker statistical tests, by means of simulated samples.

Methods

Two-Locus Segregation Models

Throughout this paper, we will assume that there are two trait loci, both of them being (1) diallelic, (2) in Hardy-Weinberg equilibrium, (3) unlinked to each other, and (4) in linkage equilibrium. Locus 1 has alleles *A* and *a* with frequencies p_1 and q_1 $(=1-p_1)$, respectively. At locus 2, allele *B* occurs with frequency p_2 and allele *b* with q_2 $(=1-p_2)$. The genetic model is completely described by specification of the penetrance for each of the nine possible two-locus genotypes. Let f_{ij} denote the penetrance for the genotype with *i* copies of allele *A* at locus 1 and with *j* copies of allele *B* at locus 2.

The penetrance patterns $(f_{ij})_{i,j=0,1,2}$ for 12 models considered in the present paper are given in table 1. The epistatic models Ep-1–Ep-6 and the heterogeneity models Het-1–Het-3 have been discussed by Neuman and Rice (1992), who also provide examples of traits and/or diseases for which these models may be applicable. The heterogeneity models S-1 and S-2 and the epistatic model S-3 have been investigated recently by Schork et al. (1993). Note that S-2 is a special form of the more general model Het-2 and is obtained by setting $\phi_2 = \phi$ and $\phi_1 = 1$ for the parameters of Het-2.

For the determination of parameter values we followed Schork et al. (1993) in choosing allele frequencies and penetrance values resulting in a trait prevalence (K_p) of .1, an offspring risk (K_{po}) of .3, and equal contribution to the trait prevalence of the two loci. Additionally, we assumed equal penetrances ($\phi_1 = \phi_2$) of the two disease causes in models Het-1–Het-3.

With the exception of Ep-2, Ep-4, Het-2, and S-2, all models are symmetrical in the two loci. For symmetrical models, equal contribution of each locus requires equal allele frequencies at the two loci. Formulas for the population-risk characteristics given in Neuman and Rice (1992) were applied to give parameter values satisfying $K_p = .1$ and $K_{po} = .3$. However, there is no such solution for models Ep-6 and Het-3. For these models, the penetrance was set to its maximum value and the allele frequency was chosen to give $K_{po}/K_p = 3$. For the asymmetrical models, the meaning of "equal contribution of each locus to trait prevalence" (Schork et al. 1993, p. 1128) is rather less obvious. For example, the interpretation of this condition by Schork et al. (1993), in the context of their model S-2, results in $p_1^2 \cdot q_2^2 = \phi \cdot (1-p_1^2) \cdot (1-q_2^2)$. An alternative (and at least equally justified) interpretation is to require that the two risk factors (*AA* at locus 1 and *BB* or *Bb* at locus 2) have equal attributable contribution to the disease. The two risk factors act independently in model S-2, so this interpretation results in $p_1^2 = \phi \cdot (1-q_2^2)$, which algebraically is not equivalent to the relationship required by Schork et al. (1993), although its solution ($p_1 = .227$; $p_2 = .046$; and

Table 2

Distribution of IBD Scores ($\theta_1=\theta_2=.0$)

Model	Joint Distribution[a]									Marker 1[b]			Marker 2[c]		
	s_{22}	s_{21}	s_{20}	s_{12}	s_{11}	s_{10}	s_{02}	s_{01}	s_{00}	$s_{2.}$	$s_{1.}$	$s_{0.}$	$s_{.2}$	$s_{.1}$	$s_{.0}$
Ep-1	.1394	.1816	.0524	.1816	.2365	.0682	.0524	.0682	.0197	.3734	.4863	.1403	.3734	.4863	.1403
Ep-2	.1476	.1901	.0529	.1772	.2281	.0635	.0531	.0684	.0191	.3906	.4688	.1406	.3779	.4866	.1355
Ep-3	.1617	.1866	.0538	.1866	.2153	.0621	.0538	.0621	.0180	.4021	.4640	.1339	.4021	.4640	.1339
Ep-4	.1538	.1460	.0395	.2182	.2025	.0460	.0925	.0846	.0169	.3393	.4667	.1940	.4645	.4331	.1024
Ep-5	.1522	.1733	.0572	.1733	.2289	.0694	.0572	.0694	.0191	.3827	.4716	.1457	.3827	.4716	.1457
Ep-6	.1731	.2007	.0865	.2007	.1446	.0479	.0865	.0479	.0121	.4603	.3932	.1465	.4603	.3932	.1465
Het-1	.1062	.1678	.0628	.1678	.2477	.0822	.0628	.0822	.0205	.3368	.4977	.1655	.3368	.4977	.1655
Het-2	.1227	.1911	.0695	.1628	.2196	.0590	.0698	.0872	.0183	.3833	.4414	.1753	.3553	.4979	.1468
Het-3	.1659	.2007	.0876	.2007	.1473	.0490	.0876	.0489	.0123	.4542	.3970	.1488	.4542	.3970	.1488
S-1	.1078	.1685	.0619	.1685	.2477	.0815	.0619	.0815	.0207	.3382	.4977	.1641	.3382	.4977	.1641
S-2	.1336	.2241	.0915	.1420	.2014	.0613	.0567	.0726	.0168	.4492	.4047	.1461	.3323	.4981	.1696
S-3	.1250	.1756	.0592	.1756	.2368	.0744	.0592	.0744	.0198	.3598	.4868	.1534	.3598	.4868	.1534

[a] s_{ij} = probability that two affected sibs share i marker alleles IBD at the first marker locus and j marker alleles IBD at the second marker locus.
[b] $s_{i.}$ = probability that two affected sibs share i alleles IBD at the first marker locus ($s_{i.}=s_{i2}+s_{i1}+s_{i0}$).
[c] $s_{.j}$ = probability that two affected sibs share j alleles IBD at the second marker locus ($s_{.j}=s_{2j}+s_{1j}+s_{0j}$).

ϕ = .573) is numerically nearly identical. In model Ep-2, there are two necessary conditions for being at risk. Both of these conditions are equally frequent if $p_1^2 = 1 - q_2^2$. In model Ep-4, we required that $P(AABb) = P(AaBB, aaBB)$, which gives $p_1^2 = p_2/(1+q_2)$. Finally, for model Het-2, $p_1^2 = 1 - q_2^2$ is required. For each model, the resulting allele frequencies and penetrance values are shown in table 1, together with the population risk characteristic induced by these values.

Induced Distribution of IBD

We assume that each of the two trait loci is linked to a marker locus at recombination fraction θ = .0. This assumption seems to be not too unrealistic in view of the availability of an increasingly dense map of markers. Moreover, the potential advantage of two-marker analysis over single-marker analysis can be expected to be most pronounced for completely linked markers.

Let s_{ij} denote the probability that two affected sibs share i marker alleles IBD at the first marker locus and share j marker alleles IBD at the second marker locus. For single-locus disease models, general formulas for the induced distribution of IBD scores in terms of allele frequencies, penetrances, and recombination fraction are easily obtainable (Suarez et al. 1978). Whereas such general formulas tend to be quite cumbersome for two-locus disease models (Hodge 1981), the numerical calculation of the distribution of IBD scores is straightforward. Table 2 presents this distribution for each of the 12 two-locus disease models considered.

We simulated samples of affected sib pairs according to the multinomial distributions given in table 2. A random-number generator proposed by Wichmann and Hill (1982) was used. Sample sizes (n) were 20–100. For each disease model and each sample size, 10^6 replicated samples were simulated.

Analysis Strategies

Let $(s_{ij}^0)_{i,j=0,1,2}$ denote the joint distribution of IBD scores in affected sibs in the case that both marker loci are unlinked to the disease; that is, $s_{ij}^0 = (\frac{1}{2})^{2+|i-1|+|j-1|}$. In total, we considered eight different statistical procedures to decide between H_0: $(s_{ij})_{i,j=0,1,2} = (s_{ij}^0)_{i,j=0,1,2}$ and H_1: $(s_{ij})_{i,j=0,1,2} \neq (s_{ij}^0)_{i,j=0,1,2}$. The nominal significance level was α = .0001 for each considered test. This roughly corresponds to the significance level of a maximum lod score of 3.0, which is traditionally employed in classical linkage analysis (Ott 1991).

If both parents possess a different heterozygous genotype at each marker locus, then for each sib pair the number of marker alleles IBD can be determined unambigously. Let n_{ij} (i, j=0, 1, 2) denote the observed number of sibs sharing i and j marker alleles IBD at the first and second marker locus. $n_{i.}$ (i=0, 1, 2) and $n_{.j}$ (j=0, 1, 2) are the marginals of this observed distribution.

The first two statistical tests we considered use each marker locus separately and reject H_0 if there is evidence for linkage for at least one marker locus. In other words, H_0 is rejected if at least one of the null hypotheses H_0^1: $(s_{0.}, s_{1.}, s_{2.}) = (\frac{1}{4}, \frac{1}{2}, \frac{1}{4})$ and H_0^2: $(s_{.0}, s_{.1}, s_{.2}) = (\frac{1}{4}, \frac{1}{2}, \frac{1}{4})$ can be rejected at a significance level α^*. If both marker loci are unlinked to the disease, the observed numbers of markers IBD at each locus are independent. Therefore, to assure that the global significance level does not exceed α, each single comparison has to be performed with $\alpha^* = 1 - \sqrt{1-\alpha}$. As has been mentioned in the

Introduction, different statistical tests have been proposed for single-marker IBD data on affected sibs. There is no test that is uniformly most powerful for all possible alternatives. But when we compared the power of the T_{max} test of Schaid and Nick (1990) with that of the tests of Faraway (1993) and Holmans (1993), we found (authors' unpublished data) that the power differences are slim over a wide range of considered alternatives. Faraway's test seems to be, on average, slightly more powerful. For any specific alternative, the most powerful test can be easily calculated (Knapp 1991). The difference in power between the test that is optimal in a given situation and a test that does not rely on the knowledge of the alternative can be regarded as the price for not knowing the underlying mode of inheritance. We observed that the maximum value of this difference was smallest for Holman's test. Therefore, we applied this test and the unrestricted likelihood-ratio test in the current context.

One-sided, unrestricted single-marker likelihood-ratio test (T1).—For the first marker locus, this test is based on

$$T_1^1(n_{0.}, n_{1.}, n_{2.}) := 2 \ln\left(\prod_{i=0}^{2} (\hat{z}_i)^{n_{i.}} \Big/ \prod_{i=0}^{2} (z_i^0)^{n_{i.}}\right)$$

(and on an analogously defined T_1^2 for the second marker locus), with $(\hat{z}_0, \hat{z}_1, \hat{z}_2) := (n_{0.}/n, n_{1.}/n, n_{2.}/n)$ denoting the unrestricted maximum likelihood (ML) estimator for $(s_{0.}, s_{1.}, s_{2.})$ and with $(z_0^0, z_1^0, z_2^0) = (¼, ½, ¼)$ denoting the single-marker IBD distribution in the case of no linkage. Because $s_{2.} > s_{0.}$ in the case of H_1^1, we used a one-sided version of this test; that is, we declared the test to be significant if $n_{2.} > n_{0.}$ and $T_1^1(n_{0.}, n_{1.}, n_{2.}) > c(n, \alpha^*)$. Asymptotic theory predicts that the critical value $c(n, \alpha^*)$ can be approximated by the $(1-2\alpha^*)$ quantile of the χ^2 distribution with 2 df. For our rather small α^*, this approximation cannot be expected to behave very soundly for $n \leq 100$. Therefore, we preferred to calculate the exact value for $c(n, \alpha^*)$. Note that because of the discreteness of the distribution of T_1^1, it is not possible to exhaust the nominal α level completely, without relying on randomized tests. For practical reasons, we decided not to consider randomized tests. Therefore, the true size of the test usually will be smaller than its nominal size.

Restricted single-marker likelihood-ratio test (T2).—Throughout this paper, we assume that the number of marker alleles IBD can be determined unequivocally. Then, the ML estimator $\tilde{z}$ restricted to the possible triangle $\{(z_0, z_1, z_2): 2z_0 \leq z_1, z_1 \leq ½\}$ can be shown to be

$$(\tilde{z}_0, \tilde{z}_1, \tilde{z}_2) = \begin{cases} \left[\frac{n_{0.}}{2(n_{0.}+n_{2.})}, \frac{1}{2}, \frac{n_{2.}}{2(n_{0.}+n_{2.})}\right] & \text{for } 2n_{1.} > n \text{ and } n_{2.} > n_{0.} \\ \left(\frac{1}{4}, \frac{1}{2}, \frac{1}{4}\right) & \text{for } 2n_{1.} > n \text{ and } n_{2.} \leq n_{0.} \\ \left[\frac{n_{0.}+n_{1.}}{3n}, \frac{2(n_{0.}+n_{1.})}{3n}, \frac{n_{2.}}{n}\right] & \text{for } 2n_{0.} > n_{1.} \text{ and } 4n_{2.} > n \\ \left(\frac{1}{4}, \frac{1}{2}, \frac{1}{4}\right) & \text{for } 2n_{0.} > n_{1.} \text{ and } 4n_{2.} \leq n \\ \left(\frac{n_{0.}}{n}, \frac{n_{1.}}{n}, \frac{n_{2.}}{n}\right) & \text{otherwise} \end{cases}$$

for the first marker locus (and analogously for the second marker locus).

Under H_0^1, the likelihood-ratio-test statistic

$$T_2^1(n_{0.}, n_{1.}, n_{2.}) := 2 \ln\left(\prod_{i=0}^{2} (\tilde{z}_i)^{n_{i.}} \Big/ \prod_{i=0}^{2} (z_i^0)^{n_{i.}}\right)$$

for the first marker locus (and, analogously, T_2^2 for the second marker locus) is asymptotically distributed like a mixture of χ^2 distributions with 2, 1, and 0 df (Holmans 1993). The information matrix equals

$$\begin{pmatrix} 8 & 4 \\ 4 & 6 \end{pmatrix}.$$

Therefore, an approximation of the critical value is given by K satisfying the equation

$$\alpha^* = .5P(\chi_1^2 > K) + \frac{\rho}{2\pi} P(\chi_2^2 > K),$$

with $\cos \rho = \sqrt{⅔}$ (for details, compare with appendix B of Holmans [1993]). But, again, this approximation led to a liberal test, and therefore we used the exact critical value.

The remaining tests consider the information at both marker loci simultaneously.

Two-sided, unrestricted two-marker likelihood-ratio test (T3).—This test rejects H_0 if

$$T_3[(n_{ij})_{i,j=0,1,2}] := 2 \ln\left(\prod_{i,j=0}^{2} (\hat{z}_{ij})^{n_{ij}} \Big/ \prod_{i,j=0}^{2} (z_{ij}^0)^{n_{ij}}\right)$$

is greater than $\chi^2_{8,1-\alpha}$ (which is 31.8276 for $\alpha = .0001$), where $\hat{z}_{ij} := n_{ij}/n$ is the ML estimator for s_{ij}.

One-sided, unrestricted two-marker likelihood-ratio test (T4).—There are different possibilities for transforming the unrestricted likelihood-ratio test into a one-sided test. We considered a version that rejects H_0 only if the number of sibs sharing both alleles IBD at both loci exceeds the number of sibs sharing no alleles IBD at all, i.e., $n_{22} > n_{00}$ and $T_3 > \chi^2_{8,1-2\alpha}$ (=30.1359 for $\alpha = .0001$).

T3 and T4 can be expected to suffer from their large number of df. One way to circumvent the problem of estimating a large number of multinomial cell probabilities is to presuppose a certain structure for these probabilities, thereby reducing the number of parameters (Bishop et al. 1975). This procedure can prove advantagous even if the cell probabilities do not coincide with the assumed structure. A natural selection for this structure in the present context is to assume that both loci are acting multiplicatively; that is, $s_{ij} = s_{i\cdot} \cdot s_{\cdot j}$ for all $i, j = 0, 1, 2$. (Note that only models EP-1–EP-3 are really multiplicative.) The restricted and unrestricted ML estimators for $(s_{ij})_{i,j=0,1,2}$ under the assumption of the multiplicative model are the products of restricted and unrestricted ML estimators for the marginals $(s_{i\cdot})_{i=0,1,2}$ and $(s_{\cdot j})_{j=0,1,2}$. The restricted and unrestricted likelihood-ratio-test statistic then becomes the sum of the corresponding likelihood-ratio-test statistics at both marker loci.

Unrestricted two-marker likelihood-ratio test for multiplicative model (T5).—Asymptotically,

$$T_5[(n_{ij})_{i,j=0,1,2}] := T_1^1(n_{0\cdot}, n_{1\cdot}, n_{2\cdot}) + T_1^2(n_{\cdot 0}, n_{\cdot 1}, n_{\cdot 2})$$

is under H_0, distributed as a mixture of χ^2 distributions with 4, 2, and 0 df. The mixing proportions are ¼, ½, and ¼, respectively. As in the case of T1 and T2, we preferred to determine the exact critical value by calculation of the convolution of T_1^1 and T_1^2.

Restricted two-marker likelihood-ratio test for multiplicative model (T6).—For

$$T_6[(n_{ij})_{i,j=0,1,2}] := T_2^1(n_{0\cdot}, n_{1\cdot}, n_{2\cdot}) + T_2^2(n_{\cdot 0}, n_{\cdot 1}, n_{\cdot 2}),$$

it is also possible to calculate the exact critical value instead of using the approximation K satisfying

$$\alpha = \left(\frac{1}{2} - \frac{\rho}{2\pi}\right) P(\chi_1^2 > K) + \left(\frac{\rho}{2\pi}\left(1 - \frac{\rho}{\pi}\right) + \frac{1}{4}\right) P(\chi_2^2 > K) + \frac{\rho}{2\pi} P(\chi_3^2 > K) + \left(\frac{\rho}{2\pi}\right)^2 P(\chi_4^2 > K).$$

Two-marker mean test (T7).—For single-locus marker data, the so-called mean test (Blackwelder and Elston 1985) is a well-known and easy-to-apply statistic. It is straightforward to extend this test to simultaneously incorporate two-locus marker data. The test statistic then becomes the total sum of marker alleles IBD at both loci; that is,

$$T_7[(n_{ij})_{i,j=0,1,2}] := \sum_{i,j=0}^{2} (i+j) \cdot n_{ij}.$$

Because of the symmetry of the distribution of T_7 under H_0, it can be expected that asymptotic approximations for the determination of critical values will behave quite well even for small α. Nonetheless, we again preferred to determine exact critical values.

As in the case of single-locus marker data (Knapp 1991), the test statistic of the most powerful test against a specified alternative $\mathbf{s} := (s_{ij})_{i,j=0,1,2}$ can easily be shown to be a linear combination of $(n_{ij})_{i,j=0,1,2}$, with weights depending on the alternative $\mathbf{s}$; that is,

$$T^{\mathbf{s}}_{\text{opt}}[(n_{ij})_{i,j=0,1,2}] := \sum_{i,j=0}^{2} c_{ij} \cdot n_{ij},$$

with $c_{ij} := \ln(s_{ij}/s_{ij}^0)$. To determine the critical value of this optimal test proves to be quite difficult. Whereas it is straightforward to calculate the mean and variances of $T^{\mathbf{s}}_{\text{opt}}$ under H_0, we observed that critical values obtained by the normal approximation of the distribution of $T^{\mathbf{s}}_{\text{opt}}$ under H_0 are of no practical value, because for most of the considered models they led to an extremely conservative test. On the other hand, in general it seems intractable to determine the critical values by calculating the exact distribution of $T^{\mathbf{s}}_{\text{opt}}$. This approach would require evaluation of $T^{\mathbf{s}}_{\text{opt}}$ for each possible sample $(n_{ij})_{i,j=0,1,2}$. For sample size n, the total number of different samples is $\binom{n+8}{n}$, which is $\approx 3.52 \cdot 10^{11}$ for $n = 100$. But this complexity is reduced for *symmetrical* models (i.e., $s_{ij} = s_{ji}$ for all $i, j = 0, 1, 2$). Remember, all but EP-2, EP-4, Het-2, and S-2 of the 12 models in table 2 are symmetrical. Since we have $c_{ij} = c_{ji}$ for symmetrical models, it is then possible to collapse the nine different cells into just six cells, thereby reducing the number of different samples to $\binom{n+5}{n}$, which is $\approx 9.66 \cdot 10^7$ for $n = 100$ and therefore is within the limits of feasibility. Each of the nonsymmetrical models was approximated by a symmetrical one by simply substituting s_{ij} with $(s_{ij}+s_{ji})/2$.

Results

True Type I Error

Figure 1*a–g* presents the estimated true type I error probabilities for the analysis strategies T1–T7. These estimators are based on the relative frequency of significant samples, of 10^6 simulated replications for each sample size n. The agreement between the nominal type I error rate of $\alpha = .0001$ (dotted line) and the estimated true type I error rate is quite good for the unrestricted (T1) and restricted (T2) single-marker likelihood-ratio tests as well as for the unrestricted (T5) and restricted (T6) two-marker likelihood-ratio tests, for the multiplicative model. However, it is also clearly visible that the determination of critical values for the two-sided (T3) and one-sided (T4) unrestricted two-marker likelihood-ratio tests leads to an anticonservative test; that is, for sample sizes >30 sib pairs, both tests possess a true type I error rate, which is clearly >.0001. For the two-marker mean test (T7), the difference between its true and its nominal type I error rate reflects the fact that this statistic has a rather low resolving power (there are only $4n + 1$ different possible values for this test statistic), and therefore it is quite impossible to exhaust the predescribed α level without resorting to randomized tests.

The true type I error of T^s_{opt} belonging to the IBD distributions of table 2 is shown in figure 2*a–l*. For all the symmetrical models, the agreement with the nominal α level of .0001 is quite satisfying. The same holds true for the nonsymmetrical models Ep-2 and Ep-4, whereas for Het-2 the predescribed α level seems to be incompletely exhausted. For S-2, there is a clear indication that the simple approximation we used resulted in an anticonservative procedure.

At this point, it seems necessary to resolve what apparently is a contradiction in the results presented so far. Knapp et al. (1994) have shown for single-marker-locus data that the mean test is optimal in the case of a recessive disease. With a similar argument it can be proved that for two-marker-locus data, the two-marker mean test is optimal if both disease loci are recessive and equally contribute to the disease. But this is exactly the disease model EP-3. Therefore, the optimal test for EP-3 and the mean test should be identical. A comparison of figure 1*g* (true error rate for the mean test) with figure 2*c* (true error rate for the optimal test against EP-3) reveals that there are differences. The explanation for these differences is that the weights of the optimal test were calculated using the values $(s_{ij})_{i,j=0,1,2}$ presented in table 2. However, these values are necessarily only rounded values. Whereas the magnitudes of the differences between these rounded values and the "true" s_{ij} are rather small, these small differences are sufficient for the "optimal" test belonging to the rounded values to distinguish between samples that have identical values by the mean test. Therefore, the "optimal" test better exhausts the nominal α level.

Power

The power of the different analysis strategies for the 12 genetic models is shown in figures 3–5. From these figures, the answer to the first question formulated in the Introduction is quite obvious. There is indeed a gain in power by considering both marker loci simultaneously. This gain can neither be obtained by using the two-sided (T3) nor the one-sided (T4) two-marker likelihood-ratio test. Despite their inflated size (fig. 1*c* and *d*), the performance of both of these tests is still rather poor. However, T5–T7 clearly show an increase in power, compared with single-marker analysis. This superiority is most pronounced for the multiplicative models Ep-1, Ep-2, and Ep-4, as could be expected in theory. But even for the remaining nonmultiplicative models, generally there is a clear visible gap between the power curves for T5–T7 and the curves belonging to the other tests. The only exception is Ep-4, for which T2 performs nearly quite as well as do T5 and T7.

For each model, T5 is less powerful than are T6 and T7. Only for Ep-4 is there a clear advantage of T6 over T7. For models Ep-6, Het-3, and S-2, the tests T6 and T7 have quite similar power curves, whereas for the remaining models T7 is more powerful than T6. In summary, T7 shows the best performance of all considered tests, which then answers the second question posed in the Introduction.

A comparison of the power curve of T7 and the power curve of the optimal test for each model shows that the range of a possible improvement in power is generally rather small. The exceptions are models Ep-4 and S-2. However, for model S-2 we have to remember that the optimal test is too liberal (fig. 2*k*).

Discussion

For mapping diseases governed by two unlinked loci, Schork et al. (1993) have shown that parametric two-marker-locus linkage analysis can provide substantially more linkage information than can standard lod-score analysis. This motivated us to perform an analogue comparison with the affected-sib-pair method. Our findings are similar to those of Schork et al. (1993) in that there is a pronounced superiority of appropriate two-marker tests, with respect to power. Whereas the utility of parametric two-marker-locus linkage analysis is hampered by the availability of a suitable segregation model and by computational feasibility, both of these problems are nonexistent in the context of affected-sib-pair data. The two-marker mean test, which in general showed the best performance for the models considered in the present paper, can be easily calculated. The power of this test is only slightly smaller than the optimal achievable power for a given model.

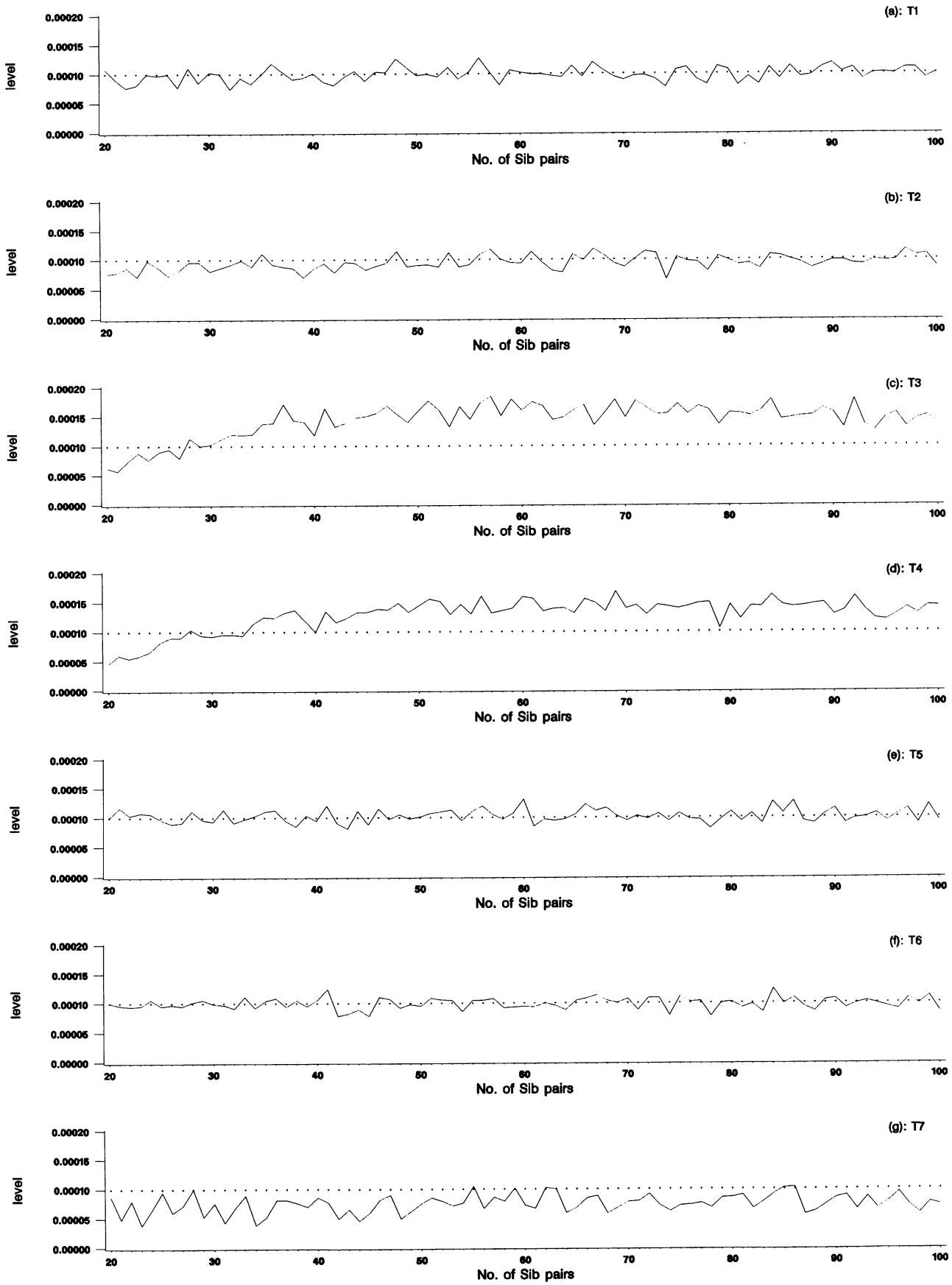

Figure 1 Simulated true type I error rate of analysis strategies T1–T7 for nominal type I error of .0001

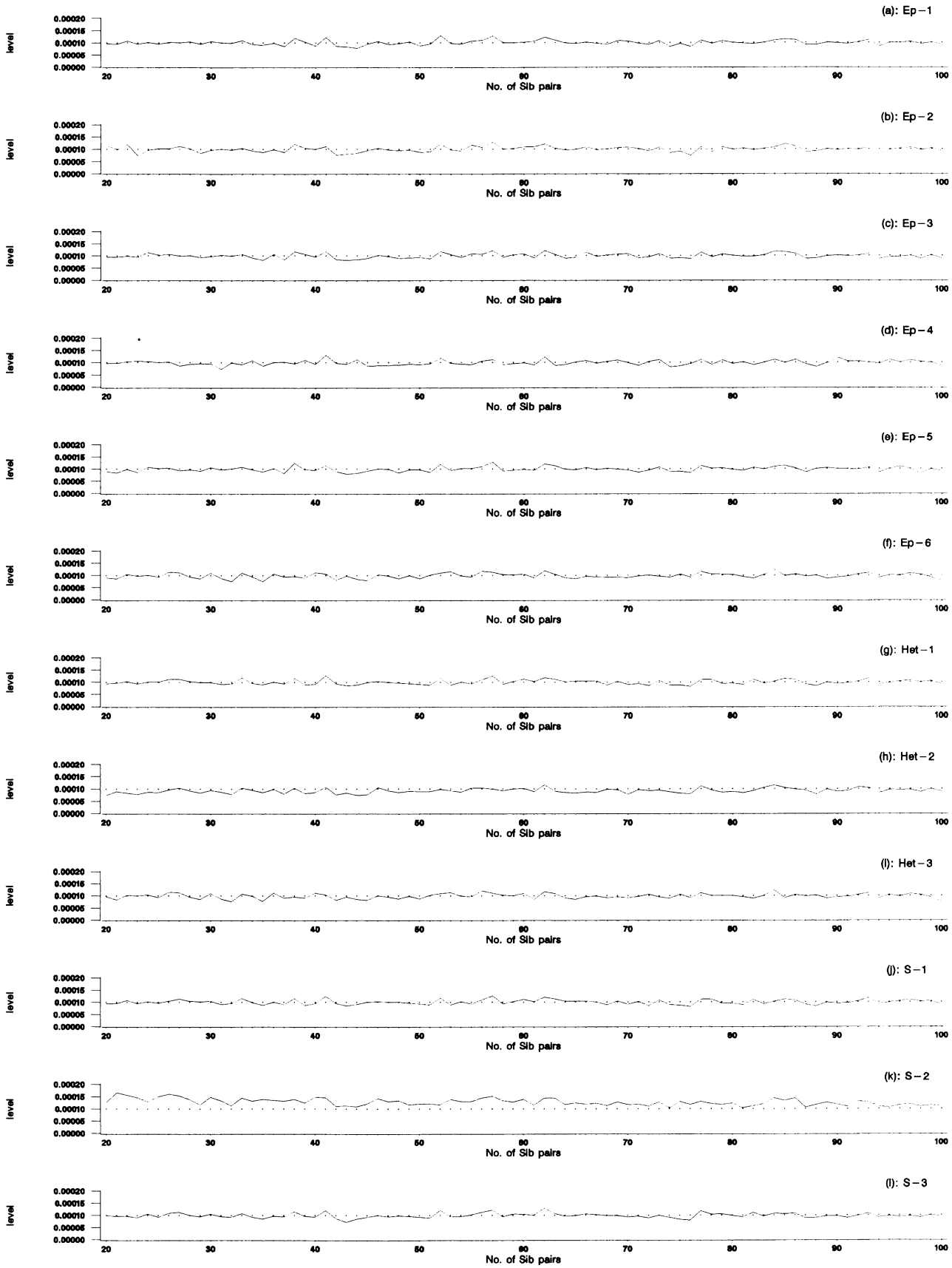

Figure 2 Simulated true type I error rate of optimal tests for models Ep-1–S-3 for nominal type I error of .0001

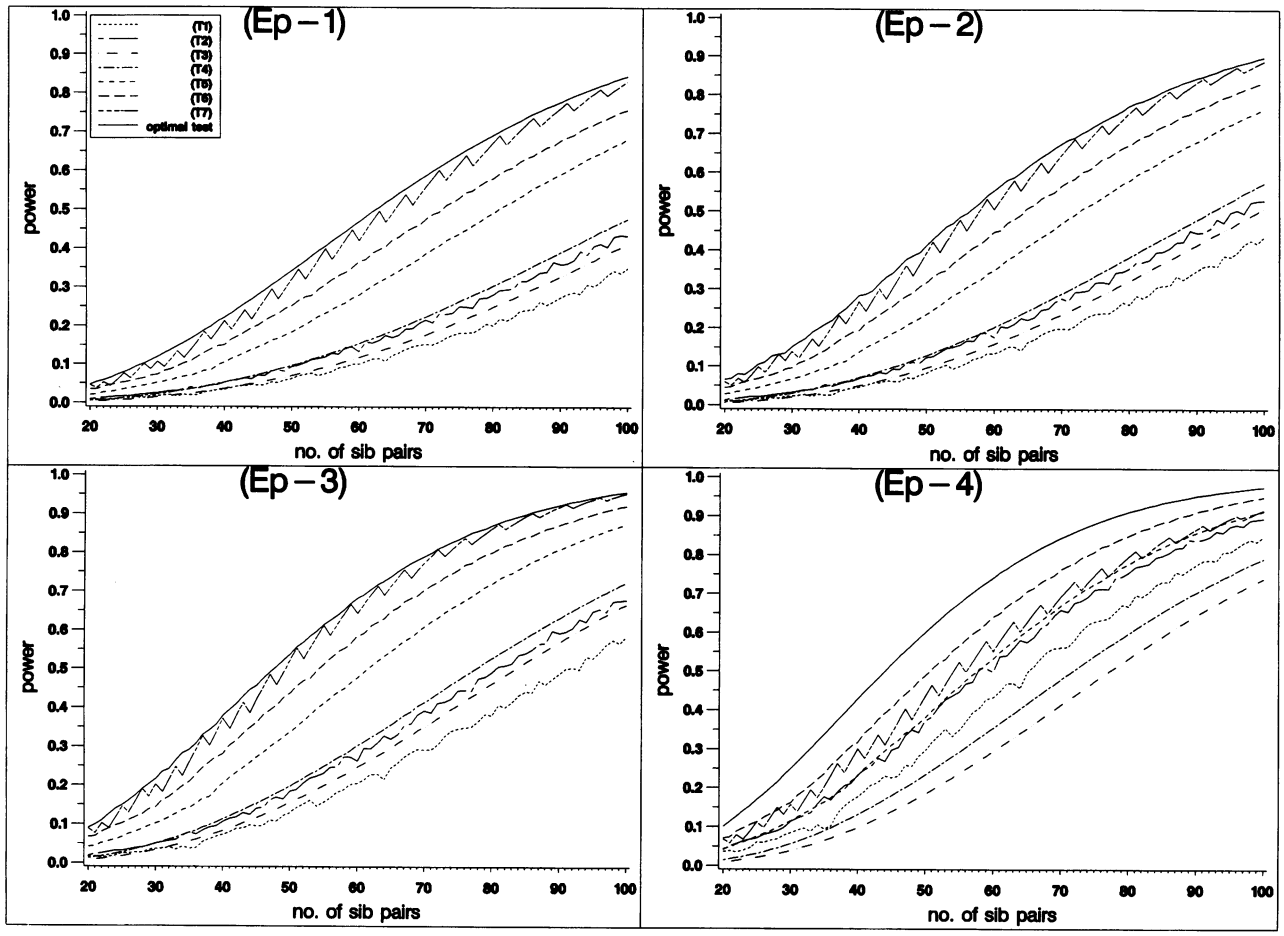

Figure 3 Simulated powers of the optimal test and of analysis strategies T1-T7 for genetic models Ep-1, Ep-2, Ep-3, and Ep-4

As described in the Methods section, the underlying assumptions for the power calculations presented in figures 3-5 are (1) that the number of marker alleles IBD can be determined unequivocally for each sib pair and (2) that each marker locus is completely linked to one of the disease loci (i.e., $\theta_1 = \theta_2 = 0$). Obviously the power will decrease if at least one of these assumptions does not hold.

When the marker is not 100% polymorphic, the investigator has the choice between two principal options. First, one can try to modify the two-marker tests proposed in the present paper so that these tests can also include sib pairs for which the IBD score is not known with certainty. For single-marker sib-pair data, this has been done by Risch (1990) and Holmans (1993). Note, however, that such a modified test necessarily relies on the marker allele frequencies. As has been clearly worked out by Babron et al. (1993), rejection of the null hypothesis by any linkage method that assumes the marker allele frequency to be known means either that there is linkage *or* that the assumed marker allele frequencies are wrong. The second option is to leave the test unchanged but to use only families for the statistical analysis for which the IBD score can be determined unequivocally. For marker loci not completely polymorphic, the set of families used for statistical analysis then will be only a subset of the families originally sampled. It is straightforward to calculate the probability that a sampled family can be used (i.e., that both parents at both marker loci are heterozygous with different genotypes), given the frequency distribution at the marker loci. For example, if each marker locus possesses $m = 6$ equally frequent alleles, this probability is $P = .42$, whereas for $m = 10$, $P = .63$, and for $m = 20$, $P = .81$.

In the Appendix, the degree of the loss of power induced by nonzero recombination fractions between marker and disease loci is quantified for the two-marker mean test. It is shown that if $\theta_1 = \theta_2 = \theta > 0$ the sample size has to be increased by roughly the factor $1/(1-2\theta)^4$, to obtain the same power as for $\theta_1 = \theta_2 = 0$. As an example, the sample size has to be increased by 17.7% if $\theta = .02$ and by 52.4% if $\theta = .05$.

What are possible further limitations of our findings? First, the major disadvantage of all simulation studies is

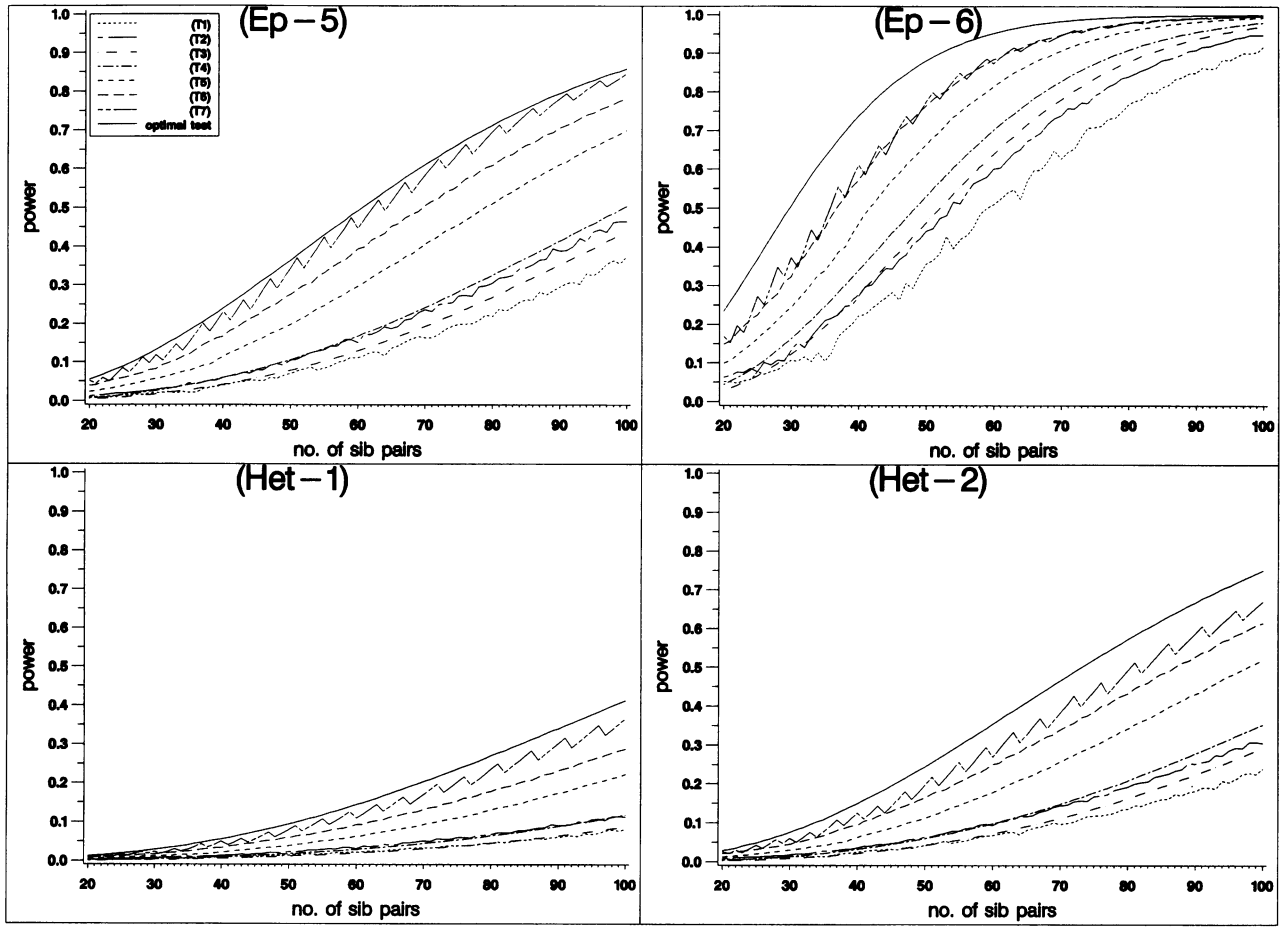

Figure 4 Simulated powers of the optimal test and of analysis strategies T1–T7 for genetic models Ep-5, Ep-6, Het-1, and Het-2

that the results are restricted to the particular situations simulated (Elston 1989). Although we believe that we evaluated the different analysis strategies for a fairly wide variety of two-locus disease models, this principal objection can never be overcome by any simulation study. Second, we exclusively devoted our attention to genetic models with two disease loci. These kinds of models acquired some popularity in the literature when the adequacy of single-locus approximations for a more complex mode of inheritance was questioned. In this context, it seems reasonable to argue that a two-locus disease model represents the worst case of all oligogenic models (Vieland et al. 1992). However, this is no longer true for the problems discussed in the present paper: If there are two marker loci considered simultaneously, a two-locus disease model represents the most favorable case of all oligogenic models. Additionally, we assumed both disease loci to contribute equally to the prevalence of the trait. Again, this assumption favors the superiority of the proposed two-marker analysis strategy. It should be quite evident that the inclusion of a second marker linked to the disease but with only a very small effect on the disease may also decrease the power of the linkage analysis. With these restrictions in mind, we nonetheless conclude that two-marker locus methods for affected-sib-pair data provide an enrichment of the arsenal of existing methodological tools for mapping genetic diseases.

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Appendix

Assume that the two-locus segregation model is fixed. Then, the induced joint distribution of IBD scores in

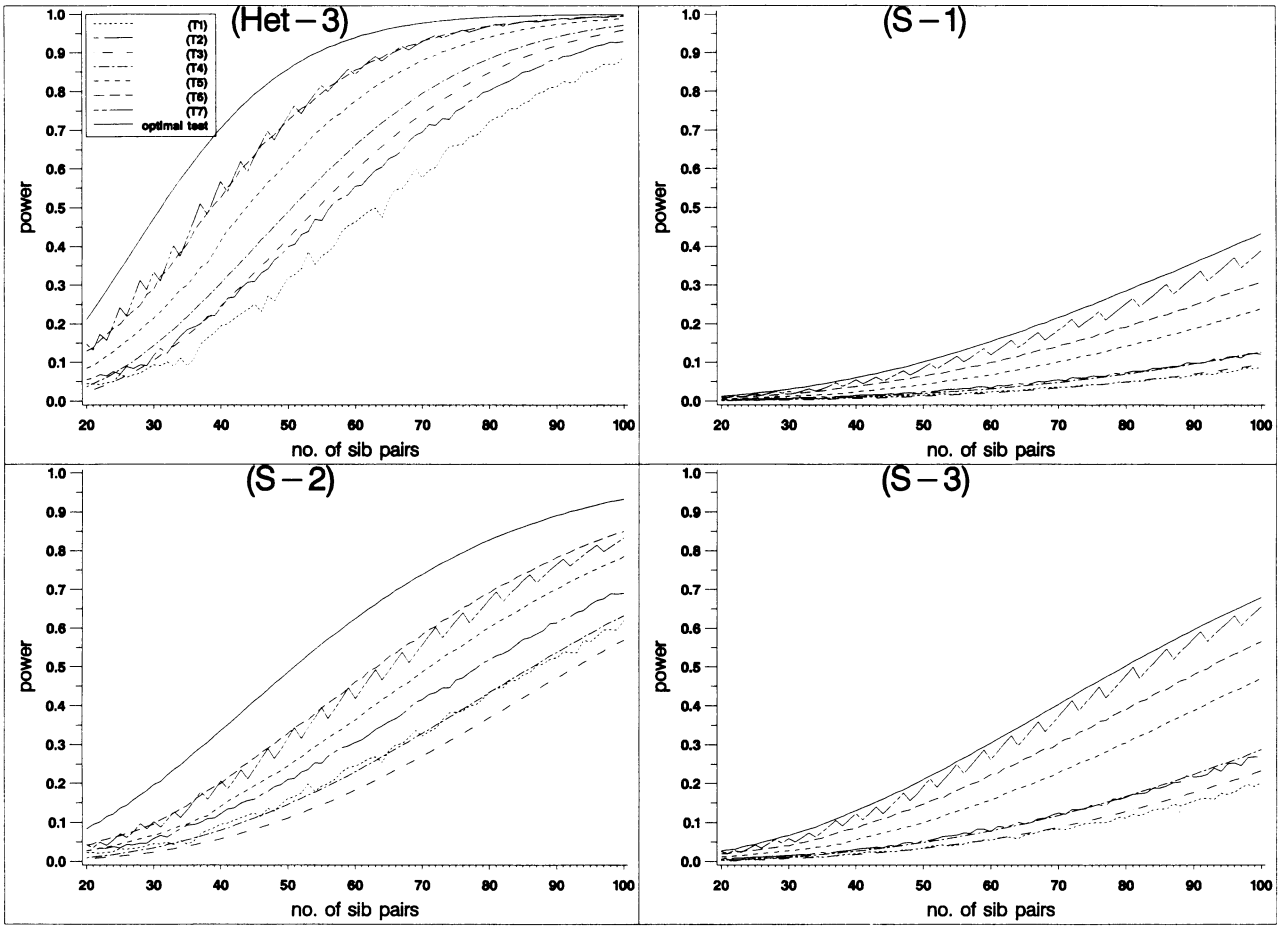

Figure 5 Simulated powers of the optimal test and of analysis strategies T1–T7 for genetic models Het-3, S-1, S-2, and S-3

affected sib pairs $\mathbf{s}(\theta_1, \theta_2) = [s_{ij}(\theta_1, \theta_2)]_{i,j=0,1,2}$ only depends on the recombination fractions θ_k between the kth disease and marker locus (k=1, 2).

In case of H_0 (i.e., $\theta_1 = \theta_2 = \frac{1}{2}$), the standardized two-marker mean test statistic

$$V_n := \frac{T_7 - E_{(\theta_1=1/2,\,\theta_2=1/2)}T_7}{\sqrt{\mathrm{Var}_{(\theta_1=1/2,\,\theta_2=1/2)}T_7}}$$

is asymptotically standard normal distributed, whereas in case of H_1, V_n is asymptotically normal distributed with

$$E_{(\theta_1,\theta_2)}V_n = \frac{E_{(\theta_1,\theta_2)}T_7 - E_{(\theta_1=1/2,\,\theta_2=1/2)}T_7}{\sqrt{\mathrm{Var}_{(\theta_1=1/2,\,\theta_2=1/2)}T_7}}$$

and

$$\mathrm{Var}_{(\theta_1,\theta_2)}V_n = \mathrm{Var}_{(\theta_1,\theta_2)}T_7 / \mathrm{Var}_{(\theta_1=1/2,\,\theta_2=1/2)}T_7 .$$

Further,

$$E_{(\theta_1,\theta_2)}T_7 = n\cdot[2s_{2.}(\theta_1, \theta_2)+s_{1.}(\theta_1, \theta_2)+2s_{.2}(\theta_1, \theta_2)+s_{.1}(\theta_1, \theta_2)] ,$$

and

$$E_{(\theta_1=1/2,\,\theta_2=1/2)}T_7 = 2n ;$$

$$\mathrm{Var}_{(\theta_1=1/2,\,\theta_2=1/2)}T_7 = n .$$

Let $\psi_k := \theta_k^2 + (1-\theta_k)^2$, $k = 1, 2$. We have (Suarez et al. 1978)

$$s_{2.}(\theta_1, \theta_2) = (1-\psi_1)^2 s_{0.}(0, 0) + \psi_1(1-\psi_1)s_{1.}(0, 0) + \psi_1^2 s_{2.}(0, 0) ,$$

$$s_{1.}(\theta_1, \theta_2) = 2\psi_1(1-\psi_1)s_{0.}(0, 0) + (\psi_1^2+(1-\psi_1)^2)s_{1.}(0, 0) + 2\psi_1(1-\psi_1)s_{2.}(0, 0) ,$$

and analogous relations for $s_{.2}(\theta_1, \theta_2)$ and $s_{.1}(\theta_1, \theta_2)$.

After some algebra, this shows

$$E_{(\theta_1,\theta_2)}T_7/n = (2\psi_1-1)(2s_{2.}(0,0)+s_{1.}(0,0)-1)+1 \\ +(2\psi_2-1)(2s_{.2}(0,0)+s_{.1}(0,0)-1)+1.$$

Now assume $\theta_1 = \theta_2 = \theta$, which implies $\psi_1 = \psi_2 = \psi$. It follows that

$$E_{(\theta_1,\theta_2)}V_n = (2\psi-1)E_{(0,0)}V_n = (1-2\theta)^2E_{(0,0)}V_n .$$

Therefore,

$$E_{(\theta_1,\theta_2)}V_{n'} = E_{(0,0)}V_n \quad \text{if} \quad n' := n/(1-2\theta)^4 . \quad \text{(A1)}$$

The variance of V_n is also affected by (θ_1, θ_2). By ignoring this dependency, equation (A1) shows that in case of nonzero recombination fraction, a sample size of $n/(1-2\theta)^4$ gives approximately the same power for the two-marker mean test as can be obtained by sample size n for completely linked markers.

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