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Direct Power Comparisons between Simple LOD Scores and NPL Scores for Linkage Analysis in Complex Diseases

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Summary

Several methods have been proposed for linkage analysis of complex traits with unknown mode of inheritance. These methods include the LOD score maximized over disease models (MMLS) and the "nonparametric" linkage (NPL) statistic. In previous work, we evaluated the increase of type I error when maximizing over two or more genetic models, and we compared the power of MMLS to detect linkage, in a number of complex modes of inheritance, with analysis assuming the true model. In the present study, we compare MMLS and NPL directly. We simulated 100 data sets with 20 families each, using 26 generating models: (1) 4 intermediate models (penetrance of heterozygote between that of the two homozygotes); (2) 6 two-locus additive models; and (3) 16 two-locus heterogeneity models (admixture α = 1.0, .7, .5, and .3; α = 1.0 replicates simple Mendelian models). For LOD scores, we assumed dominant and recessive inheritance with 50% penetrance. We took the higher of the two maximum LOD scores and subtracted 0.3 to correct for multiple tests (MMLS-C). We compared expected maximum LOD scores and power, using MMLS-C and NPL as well as the true model. Since NPL uses only the affected family members, we also performed an affecteds-only analysis using MMLS-C. The MMLS-C was both uniformly more powerful than NPL for most cases we examined, except when linkage information was low, and close to the results for the true model under locus heterogeneity. We still found better power for the MMLS-C compared with NPL in affecteds-only analysis. The results show that use of two simple modes of inheritance at a fixed penetrance can have more power than NPL when the trait mode of inheritance is complex and when there is heterogeneity in the data set.

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Introduction

Several methods have been proposed for the linkage analysis of complex traits, including maximum likelihood–based methods (LOD scores) and nonparametric approaches, such as affected sib pair (ASP) methods and the nonparametric linkage (NPL) statistic (Kruglyak et al. 1996). The maximum likelihood method uses all the data available and is the most powerful method available when the true model is used. NPL is less powerful but does not require specification of a mode of inheritance.

Although specification of a mode of inheritance appears to be a disadvantage of maximum LOD score (MLS) methods, it has been shown that LOD scores calculated with approximated genetic parameters (Mod score [Clerget-Darpoux et al. 1986], MMLS [Greenberg 1990], or MODs [Hodge and Elston 1994]) are almost as powerful as LOD scores calculated under the correct model. One can analyze linkage under two different genetic models and choose the one leading to the higher LOD score, thus increasing one's chances of detecting true linkage with a minimal cost in increased type I error (Hodge et al. 1997). We proposed that a prudent approach to linkage analysis in common disease is first to calculate LOD scores assuming two simple models, dominant and recessive, each with an arbitrary 50% penetrance, then to take the higher of the two LOD scores as the raw test statistic, and, finally, to correct for multiple tests. We call this test statistic "MMLS-C" (Greenberg et al. 1998).

However, the question of the power to detect linkage remained unanswered. In Greenberg et al. (1998), we compared the power of the MMLS-C with analysis under the true model (i.e., the generating model [GM]). Analysis under the true model is the best analysis one can expect and can be considered a "gold standard" that is unattainable for most common diseases. Using a broad range of complex genetic models (including intermediate and two-locus [2L] additive models), we showed that the MMLS-C approach usually had ~70% or greater power to detect linkage compared with the "gold standard." The relative power drops only when the power to detect linkage under the true model becomes low

Table 1

Penetrances for the Additive2 and Additive3 Models

	AA	Aa	aa
Additive2 penetrance values for:[a]			
BB	1.0	1.0	1.0
Bb	1.0	1.0	.0
bb	1.0	.0	.0
Additive3 penetrance values for:[a]			
BB	1.0	1.0	.0
Bb	1.0	.0	.0
bb	.0	.0	.0

[a] Capital letters denote disease alleles.

(<50%). That work also confirmed that, when linkage for a complex model is examined, it is the mode of inheritance *at the linked locus* being examined that is important in detection of linkage, not the overall inheritance of the disease. The inheritance at the linked locus is well approximated by a dominant or recessive model with reduced penetrance (Greenberg and Hodge 1989).

The present work focuses on the issue of power to detect linkage for MMLS-C versus NPL. Following the guidelines and results from our two previous studies, we first compare the MMLS-C approach with NPL. Second, we compare the power to detect linkage of MMLS-C with analysis under the true model in the presence of heterogeneity. (Heterogeneity models were not examined in Greenberg et al. [1998].) Third, we perform an affecteds-only comparison between MMLS-C and NPL, to remove the contribution of unaffected family members, thus comparing the power of the two methods on an equal footing, since NPL uses only affecteds. We were also interested in determining the reduction in power of MMLS-C if we used only affected individuals.

In this work we address three questions: (1) What effect does heterogeneity have on power to detect linkage by means of MMLS-C? (2) What is the power of NPL analysis, compared with MMLS-C, for intermediate, additive, and heterogeneity models? and (3) If we perform a comparison using only affected family members, how well does MMLS-C perform, compared with NPL analysis?

To answer our questions, we simulated data under a number of simple and complex models, including intermediate and 2L additive models, as in Greenberg et al. (1998), as well as 12 new 2L heterogeneity models. We (1) quantify and compare the power to detect linkage of MMLS-C versus the "gold standard" of assuming the true model in the presence of locus heterogeneity, (2) compare the power to detect linkage for MMLS-C versus NPL analysis, and (3) compare the power of both statistics for affecteds-only data when the GMs are intermediate, additive, or 2L heterogeneity.

Methods

GMs

We used a number of GMs, including all those in our previous work (Greenberg et al. 1998). We examined a total of 26 GMs: 4 single-locus intermediate, 3 2L Additive2, 3 2L Additive3, and 16 2L heterogeneity GMs. We generated family data for a single marker. We generated data sets under the following genetic models:

Intermediate Models.—In these models, the heterozygote penetrance, f_2, lies between the two homozygote penetrances, f_1 and f_3. We set $f_1 = 90\%$ and $f_3 = 0$, and then we varied f_2 over 10%, 30%, 50%, and 80%. There was always one disease locus linked to the marker with recombination fraction (θ) 0.01. The frequency of the disease allele was .01. These models are denoted Int10, Int30, Int50, and Int80, respectively.

2L Additive Models.—The Additive2 models require at least two disease alleles, total, at the two loci, for a person to be affected. One of the two disease loci is linked to the marker, with $\theta = 0.01$; the other disease locus is unlinked. The disease allele frequency at the linked locus is fixed at .01, and at the unlinked locus it is varied over 0.01, 0.05, and 0.10. Table 1 shows the penetrances for this model.

The Additive3 models require at least three disease alleles, total, at the two loci, for a person to be affected. Only one of the two disease loci is linked to the marker, as in the Additive2 models. Again, the disease allele frequency at the linked locus is fixed at .01, and at the unlinked locus it is varied over 0.01, 0.05, and 0.10. See Table 1 for the penetrances for this model.

2L Heterogeneity Models.—Heterogeneity is generated as a 2L model in which inheritance is either dominant or recessive at both loci, and penetrance is 80% or 20% at both loci. We did not generate data sets in which one locus has a dominant and the other a recessive mode of inheritance. θ for the linked locus in the heterogeneity models is also 0.01. We generated data in

Table 2

Penetrances for the Heterogeneity Models D + D and R + R

	AA	Aa	aa
D + D penetrance values for:[a]			
BB	1.0	1.0	1.0
Bb	1.0	1.0	1.0
bb	1.0	1.0	.0
R + R penetrance values for:[a]			
BB	1.0	1.0	1.0
Bb	1.0	.0	.0
bb	1.0	.0	.0

[a] Capital letters denote disease alleles; thus, in the R + R models, A and B are the recessive alleles, with frequencies q_1 and q_2, respectively.

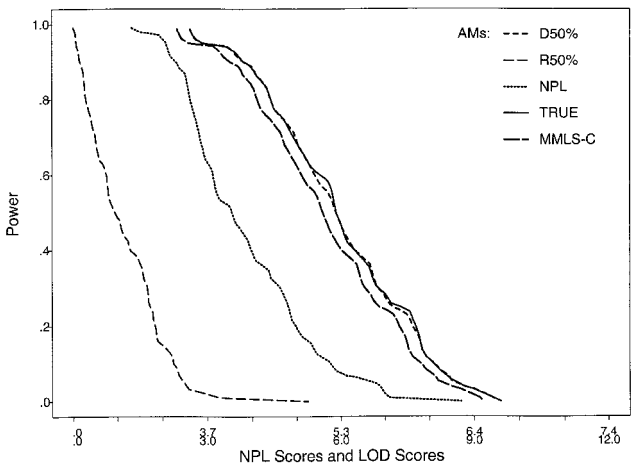

Figure 1 Power curves for D50, R50, NPL, TRUE, and MMLS-C analyses of 100 data sets generated under the Int50 model ($f_1 = .9$, $f_2 = .5$, $f_3 = 0$).

which there was linkage between the marker and 100% (H100), 70% (H70), 50% (H50), and 30% (H30) of families segregating disease in the general population. Throughout the present article, we refer to these GMs by the mode of inheritance, penetrance, and percent of families with linkage in the data set; for example, D20/H70 represents a GM with dominant mode of inheritance, 20% penetrance, and 70% families with linkage in the data set. We refer to the 2L heterogeneity models as D + D and R + R. This follows the notation of previous publications (Durner and Greenberg 1992) in which 2L heterogeneity models are referred to as D + D (i.e., D or D). This is in contrast to DD (D and D), which indicates a 2L epistatic model (Greenberg 1981). The population prevalence for all heterogeneity models was set at 1%, resulting in different gene frequencies, depending on the model. For the dominant models with linkage in 100% of families ($\alpha = 1$), the disease allele frequency was always .006. When the GM was recessive and $\alpha = 1$, the disease allele frequency was always .01. For the remaining heterogeneity models with $\alpha \leq .7$, the gene frequencies assumed single-locus analysis and were calculated as follows: $Q = k/f$, where Q represents the population frequency of at-risk genotypes, k represents population prevalence, and f is the generating penetrance. Then $Q = 1 - q_1^2 \cdot q_2^2$ for D + D models, and $Q = 1 - (1 - q_1^2)(1 - q_2^2)$ for R + R models, where, in all models, p_i is the frequency of the dominant allele and q_i is the frequency of the recessive allele at the ith locus (see table 2 for the case $f = 1$).

Data Simulation

For each of our 26 GMs, 100 data sets of 20 nuclear families each were simulated. The nuclear families were simulated according to a well-characterized family-size distribution (Cavalli-Sforza and Bodmer 1971). All matings were fully informative for the marker. Families were selected for linkage analysis if they had at least two affected children. Data sets were generated by use of our extensively tested simulation program (Greenberg 1989; Durner and Greenberg 1992; Greenberg and Doneshka 1996), which uses a random process for each step in the simulation (e.g., selecting the mating type, family size, and segregation alleles from parents to offspring). For the 2L models, we specified the penetrance of each of the nine possible genotypes.

Analysis Models (AMs)

We analyzed the simulated data for linkage, using two-point parametric and nonparametric methods. We used the following statistics:

MMLS-C Analysis.—We chose an arbitrary penetrance of 50% to analyze our data, as described in the Introduction. Misspecification of the penetrance does not generally have a strong effect on the LOD score (only on estimation of θ), as long as the dominance is specified correctly (Greenberg and Hodge 1989; Hodge and Elston 1994). We used the following algorithm:

1. Analyze under the assumption of simple dominant inheritance, with 50% penetrance (D50).
2. Analyze under the assumption of simple recessive inheritance, with 50% penetrance (R50).
3. Choose the larger of the two resultant maximum LOD score (Z_{max}) values as the MMLS score.
4. Correct for increase in type I error by subtracting 0.3 from the MMLS. The resultant score is the corrected MMLS score (MMLS-C).

Table 3

ELODs and ELOD Standard Deviations for the Intermediate and Additive GMs under Different AMs

	AM		
GM	MMLS-C	NPL	TRUE
Intermediate:[a]			
$f_2 = .1$	2.71 (1.24)	2.69 (1.20)	3.57 (1.24)
$f_2 = .3$	3.81 (1.48)	3.06 (1.09)	4.24 (1.34)
$f_2 = .5$	5.67 (1.66)	3.86 (1.43)	5.98 (1.67)
$f_2 = .8$	9.20 (1.80)	5.89 (1.69)	10.51 (2.22)
Additive with two alleles:[b]			
.01	4.13 (1.60)	3.61 (1.54)	4.97 (1.60)
.05	1.64 (1.22)	1.30 (1.05)	1.99 (1.13)
.10	.57 (.69)	.49 (.64)	1.19 (1.72)
Additive with three alleles:[b]			
.01	5.99 (2.14)	5.58 (1.75)	6.47 (2.07)
.05	3.57 (1.39)	3.21 (1.42)	4.05 (1.42)
.10	3.78 (1.45)	3.18 (1.10)	4.18 (1.36)

NOTE.—ELOD standard deviations given in parentheses

[a] Values shown are f_2; f_1 is fixed at .9; see text.

[b] Values shown are gene frequencies at the unlinked locus; see text.

Table 4

ELODs and ELOD Standard Deviations for the GMs for Affecteds-Only Analyses

	AM	
GM	MMLS-C	NPL
Intermediate:[a]		
f_2 = .1	2.93 (1.14)	2.69 (1.20)
f_2 = .3	3.56 (.99)	3.06 (1.09)
f_2 = .5	4.78 (1.36)	3.86 (1.43)
f_2 = .8	6.77 (1.44)	5.89 (1.69)
Additive with two alleles:[b]		
.01	3.45 (1.38)	3.61 (1.55)
.05	1.30 (1.04)	1.30 (1.05)
.10	.46 (.58)	.49 (.64)
Additive with three alleles:[b]		
.01	5.72 (1.89)	5.58 (1.73)
.05	3.38 (1.27)	3.21 (1.42)
.10	3.48 (1.05)	3.18 (1.10)
Homogeneity (α = 1):		
D20/H100	3.42 (1.00)	2.74 (1.14)
D80/H100	6.84 (1.58)	6.06 (2.01)
R20/H100	6.89 (1.77)	6.00 (1.53)
R80/H100	9.75 (1.36)	8.12 (1.99)
Heterogeneity (α = .7):		
D80/H70	3.15 (1.52)	2.92 (1.53)
R80/H70	5.98 (1.96)	5.13 (1.95)
Heterogeneity (α = .5):		
D80/H50	2.05 (1.28)	1.89 (1.28)
R80/H50	3.51 (1.60)	3.06 (1.55)

NOTE.—ELOD standard deviations given in parentheses.

[a] Values shown are f_2; f_1 is fixed at .9.

[b] Values shown are gene frequencies at the unlinked locus.

The LOD scores were calculated by GENEHUNTER for all single-locus models (D, R , and Int). TMLINK (Lathrop and Ott 1990) was used to calculate the "TRUE" (analysis under the true model) for the two-locus models (additive and heterogeneity). To calculate MMLS-C for the heterogeneity models, we used the maximum heterogeneity LOD score of GENEHUNTER, which is maximized over α.

NPL Analysis.—The NPL method (Kruglyak et al. 1996) can use either NPL_{all} or NPL_{pairs}, both of which use only affected individuals. We calculate only NPL_{all}. Kruglyak et al. (1996) constructed the NPL score on the basis of a score statistic (Whittemore and Halpern 1994*a*, 1994*b*). Theoretically, once the score statistic is standardized with the appropriate weights, it follows a standard normal distribution (asymptotically). The normalized NPL score for the *i*th pedigree under the null hypothesis of no linkage has mean 0 and variance 1. NPL analysis is implemented in the computer program GENEHUNTER (Kruglyak et al. 1996).

Asymptotically, the NPL scores follow a normal distribution, allowing us to transform NPL scores into LOD-score units: $(NPL)^2/(4.605) = LOD$. To confirm the assumption of normality, we plotted both the transformed NPL scores and the LOD scores on the horizontal axis of one graph. On a second graph we then plotted the exact significance levels (*P* values) obtained by GENEHUNTER as values on the horizontal axis. (Figures are not shown but are available on request.) Since all matings were fully informative for the marker, we would expect to see approximately the same power curve in both graphs. For all the GMs, the original NPL scores followed a normal distribution.

The NPL score calculated by GENEHUNTER was recognized to be conservative in the presence of missing data. Kong and Cox (1997) proposed a statistic (KAC) that has the appropriate significance level regardless of whether there is missing information. The GENEHUNTER PLUS programs implement KAC. Badner et al. (1998) showed that KAC is more powerful than the NPL score, depending on how much information is missing. In our study, we use a fully informative marker, and all family members are genotyped; therefore, the NPL has the same power as the KAC.

Affecteds-Only Analysis.—Since the NPL score uses only information from affected individuals, whereas LOD-score calculations use all available individuals in the pedigrees, we would expect some loss of power for the NPL statistic for that reason alone. Therefore, we were also interested in determining the reduction in power of MMLS-C if we used only affected individuals. To answer this question, we did a second type of comparison, coding all unaffected individuals as "unknown" and evaluating the performance of MMLS-C. We did this analysis for most of the 26 GMs, except when power to detect linkage even under the true analysis was low or when the standard deviation of the expected maximum LOD scores (ELODs) was high. (The excluded GMs were α = .70 and α = .50 at low penetrances, and all models with α = .30.)

Results

Calculation and Presentation of Power Results

We report three different test statistics: We focus primarily on the MMLS-C and the NPL in LOD-score units. In addition, all data sets were analyzed for linkage under the true model. The maximum LOD score from this analysis is reported as the TRUE score. We performed two types of analyses, including all family data and the affecteds-only analyses. For all analyses, ELODs were calculated by taking the mean of the 100 values of the particular statistic. We also calculated the standard deviations of the ELODs.

For the power calculations, the values of each statistic were ordered from highest to lowest over the 100 data sets for a given model. Observed power levels, $P(Z)$, were determined as a function of score for each test statistic T (T is a maximum Z_{max} score, NPL score, or maximum

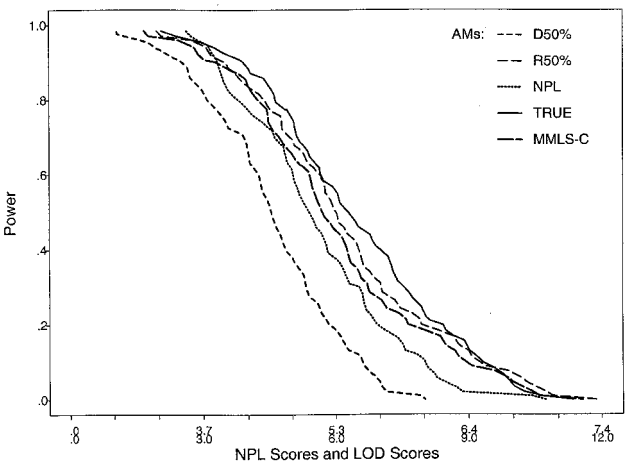

Figure 2 Power curves for D50, R50, NPL, TRUE, and MMLS-C analyses of 100 data sets generated under the Additive3 model. The unlinked gene frequency is .01.

HLOD score), as follows: $P(Z) \equiv$ (number of data sets yielding $T \geq Z$)/N, where N represents the number of data sets generated for the simulation (i.e., 100).

Figures 1–4 show selected power curves for MMLS-C and NPL, as well as curves for the corresponding D50 and R50 analyses (i.e., without correction for multiple testing) for comparison. In the graphs, power is plotted as a function of LOD scores and NPL scores. For example, if a LOD score of 3.0 (corresponding to an NPL score of 3.7) on the *X*-axis shows power of 0.8 on the *Y*-axis, this means that 80% of the 20-family data sets reached a LOD score of 3.0 or higher.

We now describe the results for the different generating models.

Intermediate Models.—For the intermediate models, as the heterozygote penetrance rises, MMLS-C consistently outperforms NPL, reaching a difference in ELODs of 3.3 LOD-score units in 20-family data sets when the GM is Int80. Table 3 presents the ELODs and the respective ELOD standard deviation for MMLS-C, NPL, and TRUE scores for the intermediate and additive GMs. (We include the TRUE results for the intermediate and additive models for ease of comparison; however, these comparisons were already made in Greenberg et al. 1998.) The NPL ELODs are almost as good as MMLS-C (ratio NPL:MMLS−C = 0.99) when the power to detect linkage was low (only 64%) even with the "gold standard" analysis, but this ratio drops to 0.64 as the information for linkage increases (table 3). For example, when the f_2 penetrance is high for intermediate models, the power to detect linkage is high. At low f_2 penetrance, the power to detect linkage is low for both methods, but, for all thresholds we examined, MMLS-C performs better than NPL. Figure 1 shows the power curves for one intermediate model, Int50, where, for a threshold of 3.0 (*P* value = .001), the power for MMLS-C is 95%, and for NPL 64%. For a threshold of 4.0 there was a drop in power of ~50% for NPL compared with MMLS-C.

Table 4 shows ELODs and standard deviations (in parentheses) for the two statistics for complex models in an affecteds-only comparison. The NPL analysis remained the same as in table 3. Table 4 shows that, for the intermediate models, the differences in ELODs between the two statistics are smaller than when all family members are included. The ELOD differences between the statistics range from 0.23 to 0.88. But as the heterozygote penetrance increases, so does the difference in ELODs of MMLS-C and NPL. Although, for the intermediate models, the unaffected individuals contribute little information for linkage, there is still an increase in ELOD of 24% under MMLS-C compared with NPL (table 4) when the GM is Int50.

2L Additive Models: Additive2 Models.—For the Additive2 model, when both loci have the same gene frequency (.01), MMLS-C gives an ELOD of 4.13, whereas NPL yields an ELOD of 3.61 (table 3). For the Additive2 model, the power to detect linkage decreases as the frequency of the disease allele at the unlinked locus increases. As the information for linkage decreases, we expect that the power for both MMLS-C and NPL will be similar, since the effect of the model assumptions on the analysis will be small. This is what we observe. When the gene frequency at the unlinked locus for the Additive2 models is .05 or .1, the ELODs for MMLS-C and NPL have similar values. This similarity arises from the fact that there is not much information for linkage in these models. The standard deviations of the ELODs for MMLS-C and NPL under these two models are relatively large (close to the corresponding ELOD). The ELODs

Table 5

ELODs and ELOD Standard Deviations for the 2L Heterogeneity GMs under Different AMs

	AM		
GM	MMLS-C	NPL	TRUE
Homogeneity (α = 1):			
D20/H100	3.17 (1.36)	2.74 (1.14)	3.88 (1.09)
D80/H100	9.35 (2.00)	6.06 (2.01)	10.72 (2.47)
R20/H100	6.89 (2.13)	6.00 (1.53)	7.48 (1.89)
R80/H100	12.47 (1.85)	8.12 (1.99)	13.52 (2.21)
Homogeneity (α = .7):			
D20/H70	1.63 (1.09)	1.60 (1.03)	1.73 (.96)
D80/H70	4.74 (1.96)	2.92 (1.53)	5.52 (2.11)
R20/H70	2.54 (1.46)	2.37 (1.50)	2.71 (1.41)
R80/H70	7.56 (2.38)	5.13 (1.95)	8.15 (2.54)
Homogeneity (α = .5):			
D20/H50	.94 (.91)	1.05 (.89)	.97 (.70)
D80/H50	3.20 (1.78)	1.89 (1.28)	3.69 (1.94)
R20/H50	1.62 (1.18)	1.53 (1.09)	1.70 (1.00)
R80/H50	4.42 (1.81)	3.06 (1.55)	4.69 (1.82)

NOTE.—ELOD standard deviations given in parentheses.

Table 6

Power to Achieve a Given *Z* Value, under the MMLS-C and True Models, and MMLS-C:TRUE (M:T) Ratio under Locus Heterogeneity

	Power to Achieve $Z = 3.0$			Power to Achieve $Z = 4.0$		
Model	MMLS-C	TRUE	M:T Ratio	MMLS-C	TRUE	M:T Ratio
Homogeneity ($\alpha = 1$):						
D20/H100	.55	.80	.69	.27	.48	.56
D80/H100	1.00	1.00	1.00	1.00	1.00	1.00
R20/H100	.98	1.00	.90	.90	.96	.94
R80/H100	1.00	1.00	1.00	1.00	1.00	1.00
Heterogeneity ($\alpha = .7$):						
D20/H70	.12	.13	.92	.04	.03	1.34[a]
D80/H70	.83	.88	.94	.56	.77	.73
R20/H70	.33	.42	.79	.16	.21	.77
R80/H70	.98	.98	1.00	.92	.95	.97
Heterogeneity ($\alpha = .5$):						
D20/H50	.03	.10	.30[a]	.01	.02	.50[a]
D80/H50	.47	.57	.83	.29	.40	.73
R20/H50	.12	.10	1.20[a]	.04	.03	1.34[a]
R80/H50	.80	.82	.98	.51	.63	.80

[a] Little information for linkage. Power under the true model ≤10%.

for the true model under these two models are also less than 3.0. Thus, the power to detect linkage under any analysis conditions is relatively low for these GMs. There is little difference in the power of NPL and MMLS-C for affecteds-only when the GMs are additive (table 4).

2L Additive Models: Additive3 Models.—When three disease alleles are required for an individual to be affected, figure 2 suggests that recessive inheritance provides a good approximation to this model with disease allele frequency at the unlinked locus .01, the same as at the linked locus (i.e., the gene frequency combination .01,.01). As the frequency of the disease allele at the unlinked locus increases to .05, we see a drop in power. At (.01,.1), the ELODs increase again. For this gene frequency combination, dominant inheritance seems to provide a better description of the inheritance model (data not shown).

The difference in ELODs between MMLS-C and NPL is not very great in this model. MMLS-C has higher ELODs by approximately half a LOD-score unit, compared with NPL, and 8% greater power to detect linkage (fig. 2). When the gene frequency of the unlinked locus is .1, the ELOD for MMLS-C is 3.78, versus 3.18 when the NPL test statistic is used, as shown in table 3. The ELODs ratio for NPL versus MMLS-C ranges from 85% to 93%.

2L Heterogeneity Models.—For the models with heterogeneity, the power to detect linkage for all analysis methods decreases as the percentage of families with linkage in the data set decreases, as expected. Also, for all α levels, as the generating penetrance increases, the MMLS-C and NPL scores increase.

The ELODs for the GMs under locus heterogeneity are presented in table 5. When the GM is D20/H100, the ELOD from MMLS-C is 3.17, versus 2.74 for the NPL statistic and 3.88 for TRUE. When the generating penetrance is 80%, the difference between MMLS-C and NPL is ~3.3 LOD-score units (table 5).

Table 6 compares the power achieved by the MMLS-C versus the TRUE statistics, at LOD-score thresholds of 3.0 and 4.0. Table 6 also shows the ratio of the MMLS-C power to the TRUE power in the presence of locus heterogeneity. The MMLS-C approach usually had ≥70% of the power to detect linkage compared with the power obtained with the TRUE analysis. When the power to detect linkage under the GM is <50%, the power of MMLS-C drops to 56%.

Table 7 presents the power to achieve *Z* of 3.0 and 4.0 by MMLS-C and NPL analyses, for all GMs. There is a corresponding difference in power of ~40% when NPL versus MMLS-C is used for the D20/H100 GMs; 55% of data sets reach a threshold of 3.0 when MMLS-C is used; 33% of data sets reach this level when NPL is used.

For the recessive models, both statistics are robust compared with the TRUE statistic. For R20/H100, when ELODs for NPL are compared with ELODs for MMLS-C, we see a ratio of 0.87 (table 5). For the GM R80/H100, there is 100% power to detect linkage if one uses MMLS-C or NPL for 3.0 and 4.0 thresholds (table 7). However, for higher heterogeneity LOD-score thresholds, MMLS-C has better power; for example, if we look at a cutoff $Z = 5.0$, the power of NPL drops to 90%, whereas, with the MMLS-C, all data sets still reach this threshold (power = 100%).

For the D80/H70 model, the ELOD for MMLS-C is 4.74 versus the 2.92 for the ELOD of NPL. Figures 3*a* and 3*b* represent the power obtained with MMLS-C and

Table 7

Power to Achieve a Given *Z* Value, for NPL, MMLS-C, NPL:MMLS-C (N:M) Ratio

	Power to Achieve $Z = 3.0$			Power to Achieve $Z = 4.0$		
Model	MMLS-C	NPL	N:M Ratio	MMLS-C	NPL	N:M Ratio
Intermediate:[a]						
$f_2 = .1$	.41	.35	.85	.16	.16	1.00
$f_2 = .3$	.71	.49	.69	.44	.21	.48
$f_2 = .5$	.95	.64	.67	.84	.40	.48
$f_2 = .8$	1.00	.95	.95	1.00	.84	.84
Additive with two alleles:[b]						
.01	.80	.58	.73	.49	.37	.76
.05	.17	.08	.47	.03	.02	.67
.10	…[d]	…[d]	…[d]	…[d]	…[d]	…[d]
Additive with three alleles:[b]						
.01	.92	.96	1.04	.85	.78	.92
.05	.63	.56	.89	.39	.30	.77
.10	.68	.56	.82	.43	.24	.56
Homogeneity ($\alpha = 1$):						
D20/H100	.55	.33	.60	.27	.12	.45
D80/H100	1.00	.94	.94	1.00	.84	.84
R20/H100	.98	.98	1.00	.90	.90	1.00
R80/H100	1.00	1.00	1.00	1.00	.99	.99
Heterogeneity ($\alpha = .7$):						
D20/H70	.12	.13	1.09[c]	.04	…[d]	…[d]
D80/H70	.83	.42	.51	.56	.21	.38
R20/H70	.33	.31	.94	.16	.18	1.10
R80/H70	.98	1.00	1.02	.92	.99	1.10
Heterogeneity ($\alpha = .5$):						
D20/H50	.03	.06	2.00[c]	.01	…[d]	…[d]
D80/H50	.47	.18	.38	.29	.06	.21
R20/H50	.12	.10	.83[c]	.04	.04	1.00
R80/H50	.80	.52	.65	.51	.26	.51

[a] Values shown are f_2; f_1 is fixed at .9.
[b] Values shown are gene frequencies at the unlinked locus.
[c] Little information for linkage. Power under the true model ≤10%.
[d] Power = 0%.

NPL for R80/H100 and R80/H70. We see that the power difference between MMLS-C and NPL decreases as α decreases. In both figures, we can see that the MMLS-C outperforms NPL.

We see a similar pattern for the H50 dominant and recessive with 80% penetrance (D80/H50 and R80/H50) models. For the low-penetrance models in which $\alpha = 0.5$, the maximum difference between MMLS-C and NPL is small and the information for linkage is low (standard deviation of ELOD ≈ 0.9). Both statistics lack power to detect linkage. All models in H30 gave small ELODs with high standard deviations and low power to detect linkage, as we expect when there is little information for linkage.

For the affecteds-only analysis, when the GM is homogeneous ($\alpha = 1$), the simple Mendelian D20 has an ELOD for MMLS-C of 3.42, versus an ELOD of 2.74 for the NPL statistic. For D80/H70 the ELOD for the true model is 3.32. The ELOD for MMLS-C was 3.15 and, for NPL, 2.92. Thus, use of NPL analysis would lead to a slight loss in power to detect linkage. When the GM is recessive with 80% penetrance under homogeneity ($\alpha = 1$) and heterogeneity ($\alpha = .7$), we observed high power for both NPL and MMLS-C in the affecteds-only analysis (fig. 4). For the affecteds-only analysis under heterogeneity, the ELODs for MMLS-C are higher than for NPL; the difference ranged from 0.2 to 0.9, as shown in table 4. We also verify that, with linkage in 50% of families, the power to detect linkage is low and MMLS-C and NPL have very close ELODs, with standard deviations of 1.28.

Discussion

The purpose of this simulation study was to answer three questions: First, how does MMLS-C perform compared with the TRUE analysis in the presence of locus heterogeneity? Second, how does the power of NPL analysis compare with the power of the MMLS-C analysis for complex models and for heterogeneity models? Third, when only affected individuals are included in the analysis, how does MMLS-C perform compared with

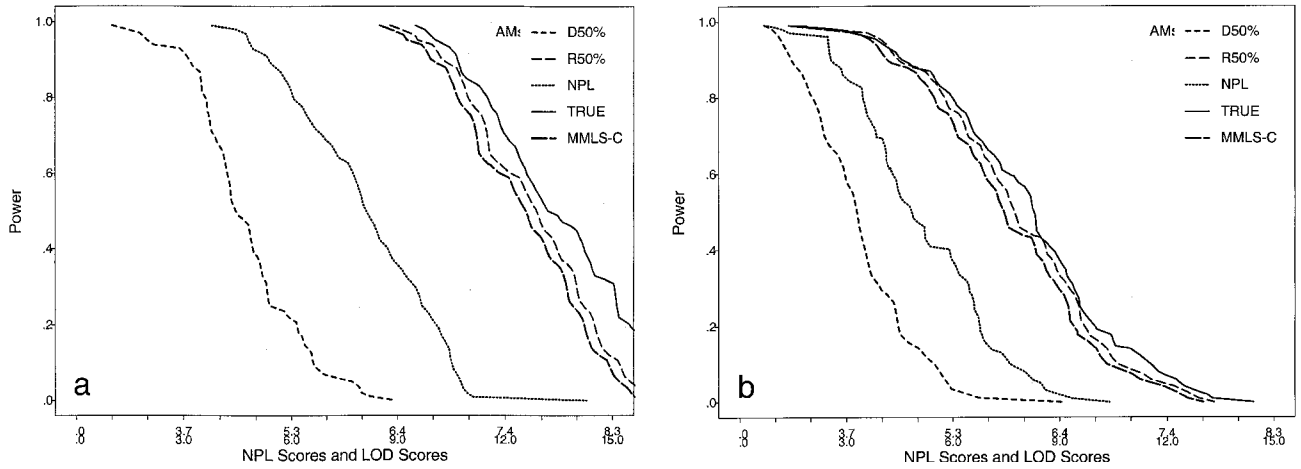

Figure 3 Power curves for D50, R50, NPL, TRUE, and MMLS-C analyses of 100 data sets generated under locus heterogeneity: *a*, GM R80, $\alpha = 1$. *b*, GM R80, $\alpha = .7$.

NPL analysis? We have shown that, for the GMs we examined, the MMLS-C approach does not substantially decrease the power to detect linkage compared with the true model, even in the presence of heterogeneity. The general pattern was that NPL had lower ELODs than the MMLS-C under all the models examined. In the presence of locus heterogeneity, as the information for linkage in a data set increases, the difference between MMLS-C and NPL increases, whether or not unaffected family members are included. MMLS-C and NPL had approximately equal power to detect linkage when there was very little information for linkage (fig. 5).

When the TRUE had power <85%, the general pattern for models under locus heterogeneity was that NPL had less power than MMLS-C. As the proportion of families with linkage increases (i.e., as α approaches 1), the difference in ELODs for MMLS-C and NPL also increases (fig. 4 and fig. 5). Performance of the MMLS-C analysis with affecteds only lowers the power of the analysis compared with analysis that includes unaffected individuals. As table 4 shows, the MMLS-C power is still higher than that of NPL for many (but not all) of the models we examined.

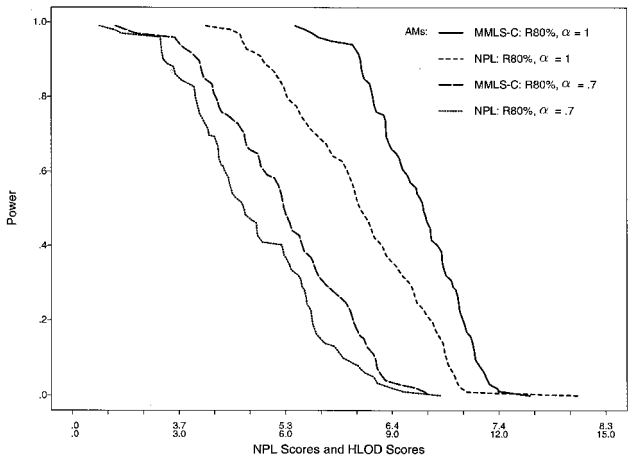

Figure 4 Power curves for MMLS-C and NPL analyses of 100 data sets generated as R80 with $\alpha = 1$ and with $\alpha = .7$ for affecteds-only analyses.

We also compared the power of MMLS-C to detect linkage when all individuals are included with the power of MMLS-C when only affected individuals are included (table 8). When penetrance was high, excluding the unaffected individuals lowered the power on average by ~25%. In the two cases (GM is Int10 or D20/H100) in which the power was slightly higher for the affecteds-only analysis, the ELODs had a large standard deviation (see table 8).

In the present study, we simulated 26 different models, to look at the power to detect linkage for MMLS-C versus NPL. In Greenberg et al. (1998), we reported only results for $\theta = 0.0$. In fact, we had also examined $\theta = 0.01$ and $\theta = 0.05$. There was no inherent difference in the behavior of MMLS-C, except that, of course, the LOD scores were higher when θ was smaller. Our focus in the present study is on the *relative* power of MMLS-C and NPL. Therefore, we did not explore the θ for this study. Also, because of the large number of models, we wanted to keep to a reasonable number of calculations.

Note that we investigated only nuclear families. Vieland et al. (1992, 1993) looked at the analysis of 2L models, using single-locus analysis for nuclear families and pedigrees. Given the results from the Vieland et al.

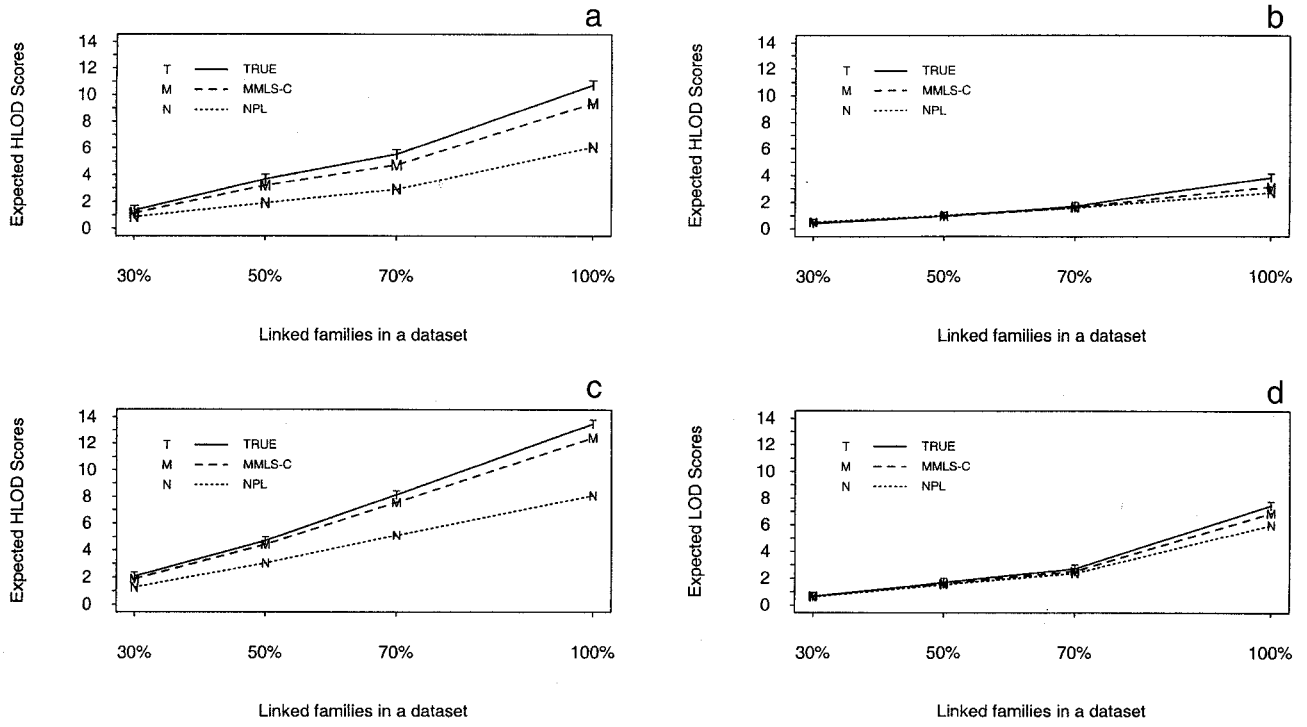

Figure 5 Expected HLOD scores for TRUE, MMLS-C, and NPL analyses of 100 data sets generated under locus heterogeneity: *a,* GM D80 at $\alpha = 1, .7, .5,$ and $.3$. *b,* GM D20 at $\alpha = 1, .7, .5,$ and $.3$. *c,* GM R80 at $\alpha = 1, .7, .5,$ and $.3$. *d,* GM R20 at $\alpha = 1, .7, .5,$ and $.3$.

studies, we would not expect fundamental differences for nuclear families versus pedigrees.

There are currently no means to incorporate heterogeneity in "model-free" analysis. Therefore, model-free methods will be weakened in their ability to detect linkage in the presence of heterogeneity. In contrast, LOD-score methods allow us to estimate α (i.e., the percentage of families with linkage in the data set). When looking at heterogeneity models, we maximized the likelihood with respect to α (HLOD), but we are simultaneously maximizing the LOD score over θ. This could be viewed as introducing another degree of freedom (Ott 1991) and therefore requiring further correction of the significance level. On the other hand, in a two-point analysis, the estimates of θ and α are highly correlated, so perhaps maximizing over α does not add a degree of freedom.

To get an idea of the distribution of the HLOD, we compared HLOD with different χ^2 curves. We simulated data sets with no linkage under two dominant and two recessive 2L heterogeneity models. Data sets were analyzed for linkage by maximizing the HLOD over dominance model. The resulting significance levels very closely matched a two-sided χ^2_1, just as the "raw" (uncorrected) MMLS curves had done (Hodge et al. 1997). (Figure not shown but available on request.) This means that, for a given type I error, an investigator would need to increase the LOD score used as a cutoff by ≈ 0.3 LOD-score units—the *same* correction as for MMLS-C; that is, an additional correction for type I error is not needed for maximization of HLOD. Therefore, since the maximum HLOD distribution follows approximately the same distribution as the maximum LOD distribution, we use the same scale and threshold LOD scores in our tables and figures.

As expected, the power to detect significant evidence of linkage is reduced in the presence of heterogeneity. We found that the power is reduced by 2%–90% (not shown), depending on both the amount of heterogeneity in the data set and the penetrance of the disease. The combination of low penetrance and even a moderate level of heterogeneity can noticeably reduce power. Figures *5a* and *5c* show that, even when the MMLS-C has more power, there is reasonably good power to detect linkage for both MMLS-C and NPL when penetrance is high. Figures *5b* and *5d* show that the power to detect linkage is low when $\alpha \leq 70\%$ and there is low penetrance. Figures *5a* and *5c* also show that, as the percent of families with linkage in the data set increases, so does the difference in the power to detect linkage for MMLS-C and NPL.

In complex diseases, in which the trait may be influenced by several different loci, at each locus, either one

Table 8

Comparison of MMLS-C ELODs for the GMs when Unaffected Individuals Are Included and for Affecteds-only Analyses

	ELODs		
GM	Affecteds Only	All	Affecteds:All
Intermediate:[a]			
f_2 = .1	2.93[c]	2.71[c]	1.08
f_2 = .3	3.56	3.81	.94
f_2 = .5	4.78	5.67	.84
f_2 = .8	6.77	9.20	.74
Additive with two alleles:[b]			
.01	3.45	4.13	.84
.05	1.30	1.64	.80
.10	.46	.57	.81
Additive with three alleles:[b]			
.01	5.72	5.99	.96
.05	3.38	3.57	.95
.10	3.48	3.78	.92
Heterogeneity (α = 1):			
D20/H100	3.42[c]	3.17[c]	1.08
D80/H100	6.84	9.35	.74
R20/H100	6.89	6.89	1.00
R80/H100	9.75	12.47	.78
Heterogeneity (α = .7):			
D80/H70	3.15	4.74	.67
R80/H70	5.98	7.56	.79
Heterogeneity (α = .5):			
D80/H50	2.05	3.20	.64
R80/H50	3.51	4.42	.79

[a] Values shown are f_2; f_1 is fixed at .9.
[b] Values shown are gene frequencies at the unlinked locus.
[c] Large standard deviation of ELOD $\cong$ 1.2.

or both alleles contribute to the trait expression, thus approximating either dominant or recessive inheritance at the specific locus. Our previous work (Greenberg et al. 1998) and several other studies (Greenberg 1989; Vieland et al. 1992; Goldin and Weeks 1993; Durner et al. 1999) showed that the important assumption in the analysis is the mode of inheritance at the specific disease locus being analyzed. The action of the other locus can be incorporated into the reduced penetrance (Greenberg and Hodge 1989). Greenberg and Berger (1994) investigated the reliability of a method for determining the mode of inheritance from the linkage data. The method examined the difference between the maximum LOD scores calculated under the dominant and recessive AMs. They showed that, if this difference was $\geq$1.5, then the higher of the two maximum LOD scores reflected the correct mode of inheritance with high reliability. A difference of $\geq$2.5 essentially guarantees a correct mode of inheritance inference. Therefore, one can gain knowledge about the mode of inheritance of the disease, using the MMLS-C approach.

For the Additive3 model, we saw that the gene frequency at the unlinked locus determined which assumed mode of inheritance at the linked locus led to the higher LOD score. When the model at the linked locus is misspecified, the LOD score drops, leading to a loss of power to detect linkage. In Durner et al. (1999), the authors carefully examined the work of Dizier et al. (1996). Dizier et al. (1996) analyzed linkage data from complex inheritance both with ASP and with LOD scores. But the LOD-score analysis used genetic parameters derived from a segregation analysis. Dizier et al. (1996) concluded that there are certain models in which ASP analysis has more power to detect linkage than LOD scores. However, Durner et al. (1999) showed that, had Dizier et al. (1996) used the MMLS-C analysis instead of using parameters from a segregation analysis, they would have had more power to detect linkage using LOD scores than either ASP or NPL.

Various studies have compared the power of different linkage methods with the NPL statistic. Lin et al. (1997) evaluated the performance of NPL under single Mendelian models and models with heterogeneity and concluded that, under a model with a major gene effect, likelihood-based methods (MMLS) tend to be more powerful. However, for a minor gene effect, the NPL statistic is generally superior to the other tests. Davis and Weeks (1997) also examined a variety of statistics for linkage analysis with different GMs and family structures. They showed that NPL had lower power compared with other methods when there was heterogeneity in the data and when families were ascertained through two or more affected children.

We have focused on two-point parametric versus two-point "model-free" analysis. We looked at some specific GMs (one-locus and 2L), trying to cover a broad range of genetic models. We concluded that MMLS-C has better power than NPL under the range of GMs we examined. Our intention was to show that parametric methods remain a powerful tool even when the underlying genetic model is unknown. However, there might be circumstances in which model-free methods will be better suited for a genetic analysis than the parametric methods. Our current work in preparation looks into power comparisons between MMLS-C and NPL for multipoint analysis for the same GMs. Our results thus far demonstrate that the conclusions of this work apply equally well to multipoint analysis.

We conclude that

1. Our proposed statistic MMLS-C is simple and robust, and its power to detect linkage is often almost as great as that obtained with the true model.
2. MMLS-C has more power than NPL for complex models.
3. MMLS-C yields better power to detect linkage than NPL under heterogeneity ($\alpha \neq 1$).
4. MMLS-C has more power than NPL when only affecteds are analyzed. For the affecteds-only analysis,

the MMLS-C was uniformly more powerful than NPL for most of the cases we examined.

5. As information for linkage goes down, so does the difference between MMLS-C and NPL.

6. When only affected family members were analyzed, the expected LOD score was on average ~25% lower than when we included the unaffected family members.

7. The inheritance at one locus approximates either dominant or recessive inheritance.

An advantage of MMLS-C is that it provides information about the mode of inheritance at the locus being tested, whereas NPL does not. The results show that our approach, using two simple modes of inheritance at a fixed penetrance, can have more power than NPL when the trait mode of inheritance is complex and in the presence of locus heterogeneity. Mendelian models, despite their simplicity, provide a reasonable approximation for a locus-by-locus search for disease genes.

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References

Badner JA, Gershon ES, Goldin LR (1998) Optimal ascertainment strategies to detect linkage to common disease alleles. Am J Hum Genet 63:880–888

Cavalli-Sforza LL, Bodmer WF (1971) The genetics of human populations. Freeman, San Francisco

Clerget-Darpoux FC, Bonaïti-Pellié C, Hochez J (1986) Effects of misspecifying genetic parameters in lod score analysis. Biometrics 42:393–399

Davis S, Weeks DE (1997) Comparison of nonparametric statistics for detection of linkage in nuclear families: single marker evaluation. Am J Hum Genet 61:1431–1444

Durner M, Greenberg DA (1992) Effect of heterogeneity and assumed mode of inheritance on lod scores. Am J Med Genet 42:271–275

Durner M, Vieland VJ, Greenberg DA (1999) Further evidence of increased power of LOD scores compared with nonparametric methods. Am J Hum Genet 64:281–289

Dizier MH, Babron MC, Clerget-Darpoux FC (1996) Conclusions of LOD-score analysis for family data generated under two-locus models. Am J Hum Genet 58:1338–1346

Goldin LR, Weeks DE (1993) Two-locus models of disease: comparison of likelihood and nonparametric linkage methods. Am J Hum Genet 53:908–915

Greenberg DA (1981) A simple method for testing two-locus models of inheritance. Am J Hum Genet 33:519–530

——— (1989) Inferring mode of inheritance by comparison of lod scores. Am J Med Genet 34: 480–486

——— (1990) Linkage analysis assuming a single-locus mode of inheritance for traits determined by two loci: inferring mode of inheritance and estimating penetrance. Genet Epidemiol 7:467–479

Greenberg DA, Abreu PC, Hodge SE (1998) The power to detect linkage in complex disease using simple genetic models. Am J Hum Genet 63:870–879

Greenberg DA, Berger B (1994) Using lod-score differences to determine mode of inheritance: a simple, robust method even in the presence of heterogeneity and reduced penetrance. Am J Hum Genet 55:834–840

Greenberg DA, Doneshka P (1996) The partitioned association-linkage (PAL) test: distinguishing "necessary" from "susceptibility" loci. Genet Epidemiol 13:243–252

Greenberg DA, Hodge SE (1989) Linkage analysis under "random" and "genetic" reduced penetrance. Genet Epidemiol 6:259–264

Hodge SE, Abreu PC, Greenberg DA (1997) Magnitude of type I error when single-locus linkage analysis is maximized over models: a simulation study. Am J Hum Genet 60: 217–227

Hodge SE, Elston RC (1994) Lods, wrods, and mods: the interpretation of lod scores calculated under different models. Genet Epidemiol 11:329–342

Kong A, Cox NJ (1997) Allele sharing models: lod scores and accurate linkage tests. Am J Hum Genet 61:1179–1188

Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 58:1347–1363

Lathrop GM, Ott J (1990) Analysis of complex diseases under oligogenic models and intrafamilial heterogeneity by LINKAGE programs. Am J Hum Genet 47:A188

Lin MW, Zhao JH, Curtis D, Sham P (1997) Power comparisons of nonparametric linkage tests. Am J Med Genet (Neuropsychiatr Genet) 74:601

Ott J (1991) Analysis of Human Genetic Linkage. 2d ed. Johns Hopkins University Press, Baltimore

Vieland VJ, Greenberg DA, Hodge SE (1993) Adequacy of single-locus approximations for linkage analyses of oligogenic traits: extension to arbitrary pedigree structures. Hum Hered 43:329–336

Vieland VJ, Hodge SE, Greenberg DA (1992) Adequacy of single-locus approximations for linkage analyses of oligogenic traits. Genet Epidemiol 9:45–59

Whittemore AS, Halpern J (1994*a*) Probability of gene identity by descent: computation and applications. Biometrics 50: 109–117

Whittemore AS, Halpern J (1994*b*) A class of tests for linkage using affected pedigree members. Biometrics 50:118–127