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The Power to Detect Linkage in Complex Disease by Means of Simple LOD-Score Analyses

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Summary

Maximum-likelihood analysis (via LOD score) provides the most powerful method for finding linkage when the mode of inheritance (MOI) is known. However, because one must assume an MOI, the application of LOD-score analysis to complex disease has been questioned. Although it is known that one can legitimately maximize the maximum LOD score with respect to genetic parameters, this approach raises three concerns: (1) multiple testing, (2) effect on power to detect linkage, and (3) adequacy of the approximate MOI for the true MOI. We evaluated the power of LOD scores to detect linkage when the true MOI was complex but a LOD score analysis assumed simple models. We simulated data from 14 different genetic models, including dominant and recessive at high (80%) and low (20%) penetrances, intermediate models, and several additive two-locus models. We calculated LOD scores by assuming two simple models, dominant and recessive, each with 50% penetrance, then took the higher of the two LOD scores as the raw test statistic and corrected for multiple tests. We call this test statistic "MMLS-C." We found that the ELODs for MMLS-C are $\geqslant$80% of the ELOD under the true model when the ELOD for the true model is $\geqslant$3. Similarly, the power to reach a given LOD score was usually $\geqslant$80% that of the true model, when the power under the true model was $\geqslant$60%. These results underscore that a critical factor in LOD-score analysis is the MOI at the linked locus, not that of the disease or trait per se. Thus, a limited set of simple genetic models in LOD-score analysis can work well in testing for linkage.

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Introduction

There has been discussion recently about the methods that one should use to analyze human linkage data. One school of thought is that methods that use only affected family members—methods such as affected sib pair (ASP) (e.g., see Haseman and Elston 1972; Suarez and Van Eerdewegh 1984), affected pedigree member (APM) (Weeks and Lange 1988), or nonparametric linkage (NPL) (Kruglyak and Lander 1995)—are the most appropriate methods to use. We have argued that maximum-likelihood methods, which use all the data available, often remain the most powerful and versatile methods available (Greenberg et al. 1996)

The most important perceived limitation of the maximum-likelihood or LOD-score (Z) method is that the user must specify genetic parameters—particularly the mode of inheritance (MOI)—in order to use the method. However, during the last decade, numerous investigators have shown that one can circumvent this difficulty by maximizing the maximum Z (Z_{max}) with respect to the genetic parameters (e.g., see Clerget-Darpoux et al. 1986; Elston 1989; Greenberg 1989; Clerget-Darpoux and Bonaïti-Pellié 1992; Hodge and Elston 1994). This means that, in the search for disease loci, one can analyze the data for linkage under several genetic models, then use the *highest* Z_{max} as the test statistic for linkage.

There are three legitimate concerns about this approach, which is called "MMLS" (maximized maximum LOD score) (Greenberg 1989), or "mod scores" (Clerget-Darpoux et al. 1986). First, by varying the genetic parameters or analysis model (AM), one is performing multiple tests, with a resultant increase in the probability of type I error. Second, there is an unknown effect on statistical power, as a result of correction for that increase in type I error. The third and critical concern can be phrased as a question: Even if one is willing to assume several inheritance models, is there reason to believe that any of them approximate the true MOI? For example, the true MOI might involve several loci that act in an additive fashion. What is the effect on power when one assumes a simple Mendelian model but the true model is far from simple dominant or recessive?

Recently, we partially answered the first question by quantifying the increase in type I error when testing multiple models. We showed that, if one performs a linkage analysis twice, once assuming dominant and once assuming recessive MOI, with an arbitrary penetrance of 50%, then the Z threshold for significance must be increased by, at most, ~.3 for $Z_{max} \leqslant 3.0$ (Hodge et al. 1997). This conservative correction is more stringent than necessary, in most of the cases that we examined. As a result of this work, we suggested following a simple approach to linkage analysis when the MOI is unknown, an approach designed to extract a maximum amount of information from the data while minimizing the increase in type I error: Analyze the data twice—once with dominant inheritance assumed and once with recessive inheritance assumed, each at some arbitrary penetrance, such as .5; choose the larger of the two resultant Z_{max} values; then "correct" the result by subtracting a *correction factor,* to allow for the fact that two tests have been performed. This approach represents a particularly simple version of MMLS, since it maximizes Z_{max} over only two distinct genetic models. Elsewhere, it also has been shown that, when there is linkage, the change in Z_{max} is usually relatively modest as the penetrance is varied and that relatively little information is lost by assuming a single penetrance (Greenberg 1989). However, questions of how this simple MMLS procedure affects the *power* to detect linkage remain unanswered.

This issue of power represents the focus of the present article. We address the remaining two questions mentioned above: What are the effects, on power, of (1) correcting for multiple testing and (2) assuming a simple MOI when the true MOI is complex?

We already have evidence that assuming that there are simple modes of inheritance in linkage analysis provides a robust approximation. For example, there is little effect on the Z if one assumes a single-locus model when the true MOI is a two-locus epistatic model, provided that one assumes approximately the correct MOI *at the locus linked to the marker* (Greenberg and Hodge 1989; Greenberg 1990; Vieland et al. 1992; Goldin and Weeks 1993; Hodge 1998; Leder et al. 1998). That is, if one designates dominant/recessive correctly at the linked locus, the effect of the second locus can be subsumed in "penetrance," with relatively little loss of power to detect linkage. The question remains, What happens if the true model is "intermediate" or "additive" (defined below)?

To answer our two questions, we undertook a simulation study to examine how the simple MMLS approach described above affects the power to detect linkage, under a variety of true genetic models. We (1) quantify the effect of correction for multiple testing on power and (2) examine the power to detect linkage when one assumes two simple Mendelian models for the linkage analysis but the true models are intermediate or additive.

Methods

In the present study, we are comparing the power resulting from the simple MMLS method versus that resulting from analysis under the true model—that is, a "gold standard" that represents an upper limit on Z. We are not comparing MMLS directly with any other method of linkage analysis, such as one of the affecteds-only methods. A study is currently in progress on the power of the affecteds-only methods, a study similar to what we have done here for the maximum-likelihood method.

Generating Models (GMs)

We simulated data under several different genetic models. There was always one disease locus linked to the marker with recombination fraction = .01:

1. *Dominant with 20% and 80% penetrance.* The disease-allele frequencies were always .01. These GMs are denoted "D20" and "D80."
2. *Recessive with 20% and 80% penetrance.* The disease-allele frequencies were always .01. These GMs are denoted "R20" and "R80."
3. *Intermediate (i.e., when the heterozygote penetrance,* f_2*, is between the two homozygote penetrances,* f_1 *and* f_3). Always, $f_1 = 90\%$ and $f_3 = 0$; then f_2 is varied over 10%, 30%, 50%, and 80%. The frequency of the disease allele was .01. These models are denoted "Int10," "Int30," "Int50," and "Int80." We chose the intermediate model because our other simulations showed that, whereas, in a model in which f_1 is high and $f_2 = f_3 = 0$ (i.e., a simple recessive), linkage is easy to detect, in a model in which f_1 is high but f_2 is low (say, 5%–15%) but not zero, linkage is much more difficult to detect. This was borne out in the current simulations (see Results).
4. *Additive.* These two-locus models require that the count of disease alleles at two loci reach some specified number in order for a person to be affected. Only one of the two disease loci is linked to the marker. We investigated two different sets of "additive" models. The first set, denoted "additive3," required at least three disease alleles at the two loci. The disease-allele frequency at the linked locus was fixed at .01 and that at the *unlinked* locus was varied over .01, .05, and .10. (This model resembles a two-locus epistatic model in which both loci are dominantly inherited, except that the double heterozygote is unaffected because there are a total of only two disease alleles present at the two loci.) Table 1 shows the penetrance matrix for this model. The second set of additive models, denoted "additive2," was

Table 1

Additive3

	Penetrance Value for[a]		
	AA	Aa	aa
BB	1.0	1.0	.0
Bb	1.0	.0	.0
bb	.0	.0	.0

[a] Capital letters denote disease alleles.

the same as the first, except that it required at least two disease alleles at the two loci; the disease-allele frequency at the linked locus was fixed again at .01 and that at the unlinked locus was varied again over .01, .05, and .10. Table 2 shows the penetrance matrix for this model.

Thus we examined a total of 14 GMs: 2 dominant, 2 recessive, 4 intermediate, 3 additive3, and 3 additive2 models.

Data Simulation

Data were simulated by means of a modification of our well-tested two-locus simulation program (Greenberg 1989; Greenberg and Doneshka 1996). The modification allows independent penetrance specification for each of the nine possible genotypes in a two-locus model with two alleles (disease and normal) at each locus (see below). Nuclear families were simulated according to a well-characterized family-size distribution (Cavalli-Sforza and Bodmer 1971, pp 310–313), and there had to be at least two siblings affected in order for a family to be ascertained. One thousand data sets of 20 families each were simulated for each set of generating parameters—that is, for each of our 14 GMs.

AMs

One of the objects of this study was to quantify the power to detect linkage when data are generated from "complex" models of inheritance but are analyzed on the assumption that the models are relatively simple. Therefore, the simulated data were analyzed for linkage under the assumption of simple dominant inheritance, with 50% penetrance (called "D50 analysis"), and under the assumption of simple recessive inheritance, also with 50% penetrance (R50). (This is our suggested approach, the simple MMLS approach, described above.) The resultant Z_{max} values were maximized over the "dominance model"; that is, they were maximized with respect to R50 versus D50. Thus, after analysis of a data set by use of D50 and R50, the larger of the two Z_{max} values was taken as the raw MMLS score. Then, because we were performing linkage analysis under multiple (two) models, we also corrected for the increase in type I error. When the Z is maximized over the dominance model, as here, the Z threshold for significance needs to be increased by a correction factor, or, equivalently, the test statistic itself, maximized Z_{max}, needs to be *decreased* by this correction factor, which is what we did for the present study.

In a previously published article, we showed that a correction factor of .3 appears to be conservative, for $Z_{max} \leqslant \sim 3.0$. The actual correction factor that we used depended on the Z_{max} itself (Hodge et al. 1997). In practice, to correct for multiple tests, we subtracted from the maximized Z_{max} a correction factor that varied from .24, for $Z_{max} \leqslant 0.59$, to .3, for $Z_{max} \geqslant 3.0$. This correction factor is taken from table 4 in Hodge et al. (1997). The resultant score is the "corrected MMLS score" (MMLS-C), and this is the test statistic that we report in the Results section below.

We note that the correction factor was applied separately to the maximized Z_{max} for each data set. This means that no simple relationship exists between the ELODs for MMLS-C and the ELODs for the separate D50 or R50 analyses. For GMs for which either the D50 analysis or the R50 analysis is consistently superior to the other (e.g., for the D80 GM, see table 3), the ELOD for the "raw" (uncorrected) MMLS will equal the ELOD for the superior analysis, and therefore the ELOD for MMLS-C will be ~0.3 less than the ELOD for the superior analysis. However, when the two AMs produce Z values that are relatively close to each other (e.g., for the Int10 GM), the higher Z_{max} value may, for any individual data set, occur under either AM.

Finally, all data sets were analyzed for linkage under the true model—that is, the GM. The Z_{max} from this analysis is reported as the "TRUE" score.

Thus, we calculate and report two different test statistics: MMLS-C and TRUE. Calculations were performed by means of LIPED (Ott 1974), for all the single-locus models (i.e., those with prefixes "D," "R," and "Int"), and by means of TMLINK (Lathrop and Ott 1990), for exact calculation of the Z for the additive models.

Calculation and Presentation of ELOD and Power Results

ELODs were calculated by summation of the 1,000 values of the particular test statistic and then division

Table 2

Additive2

	Penetrance Value for[a]		
	AA	Aa	aa
BB	1.0	1.0	1.0
Bb	1.0	1.0	.0
bb	1.0	.0	.0

[a] Capital letters denote disease alleles.

Table 3

ELODs for GMs under Different AMs

	AM			
GM	D50	R50	MMLS-C	TRUE
Simple Mendelian:				
D20	3.0	1.8	2.8	3.5
D80	9.5	.5	9.2	10.6
R20	5.5	10.1	9.9	10.2
R80	8.1	14.6	14.5	15.6
Intermediate:[a]				
.1	2.8	2.5	2.8	3.5
.3	4.0	1.7	3.8	4.3
.5	6.0	1.3	5.7	6.0
.8	9.7	.5	9.4	10.7
Additive3[b]				
.01	5.1	6.8	6.5	6.8
.05	3.9	3.3	3.8	4.3
.10	4.1	2.7	3.9	4.3
Additive2[b]				
.01	4.7	2.1	4.5	5.2
.05	1.7	.5	1.4	1.8
.10	.6	.3	.4	.7

[a] Values shown (i.e., .1, .3 .5, and .8) are f_2; f_1 is fixed at .9.

[b] Values shown (i.e., .01, .05, and .10) are gene frequency at the unlinked locus.

of the total by 1,000. We show these ELODs for the two test statistics being compared (MMLS-C and TRUE). (In the ELOD tables below, we also show the original D50 and R50 scores, for comparison.) We note that the ELOD for the "raw" (uncorrected) MMLS score would usually fall between the larger ELOD for the two AMs and the ELOD for the TRUE score; that is max E[*D50*,*R50*] $\leqslant$ *E*[*raw MMLS*] $\leqslant$ *E*[*TRUE*]. Since E[*MMLS-C*] $\approx$ *E*[*raw MMLS*] $-$ 0.30, we can see the following approximate relationships:

$$\max \text{E[D50,R50]} \leqslant \text{E[MMLS-C]} + 0.30 \leqslant \text{E[TRUE]}\ , \quad (1)$$

or, equivalently, approximately max E[*D50*,*R50*] $-$ 0.30 $\leqslant$ *E*[*MMLS-C*] $\leqslant$ *E*[*TRUE*] $-$ 0.30.

For the power calculations, each test statistic was ordered from highest to lowest over the 1,000 data sets, for each simulation (i.e., for each of the 14 GMs). The proportions of the 1,000 values that reached or exceeded specific Z thresholds were tabulated; these proportions correspond to power levels for those thresholds. We show these results in the tables, and we show selected power curves in the figures. In the figures, we also show power curves for the corresponding D50 and R50 analyses, for comparison. For each different type of GM, we have arbitrarily selected one or two power curves to show in the figures.

Results

1. *Dominant Models* (*D20 and D80*)

Table 3 shows the simulated ELODs calculated from data generated under the D20 and D80 models. When the generating penetrance is 20% (i.e., the GM is D20), the difference between the MMLS-C and the TRUE is 20% (.7 Z units) for D20 and 13% (1.4 Z units) for D80. The ELOD from MMLS-C is 2.8, versus 3.5 for the TRUE statistic (i.e., analyzed under D20). (For comparison, note that the ELOD is 3.0 under the dominant [D50] analysis, vs. 1.8 under the recessive [R50] analysis.) When the generating penetrance is 80% (D80), the difference is somewhat greater: ELOD for MMLS-C is 9.2, vs. 10.6 for TRUE. It is interesting to note that maximizing Z with respect to both the dominance model and penetrance would have yielded an ELOD of 10.6, since one would have encompassed the true model. The correction factor required for maximizing with respect to both the dominance model and penetrance would have been ~.6 (Hodge et al. 1997). In that case, it would yield an MMLS-C of 10.0, or 0.8 Z units higher than the statistic resulting when we fix the penetrance at the arbitrary value of 50%. Thus, in this case, maximizing $Z_{\max}$ with respect to both the dominant model and penetrance would have yielded a net increase in the ELOD after correction for maximization over both the dominance model and penetrance. For most other GMs, the cost in correction for type I error for both penetrance and the dominance model most likely would have been greater than the increased ELOD, although we did not test this.

Table 4 compares the power achieved by the MMLS-C versus the TRUE statistics, at Z thresholds of 2.0, 3.0, 4.0, and 5.0, and shows the ratio of the MMLS-C power to the TRUE power (MMLS-C:TRUE ratio). As might be expected, the maximum power loss occurs when the AM model is "farthest" from the true model, which, in this case, means the AM in which the penetrance is farthest from the true penetrance, and when there is less information for linkage in the data to begin with. When the true model (the GM) is D20, the MMLS-C:TRUE ratio varies from a low of 50%, at Z = 4.0, to a high of 79%, at Z = 2.0. Note that the lowest MMLS-C:TRUE ratio (50%) occurs only when the absolute power of both MMLS-C and TRUE is also low, well under 50%. When the GM is D80—that is, when there is a great deal of information in the data to begin with—the power levels are almost indistinguishable at all four threshold values that we examined (i.e., ~1 for both MMLS-C and TRUE). Figure 1 shows the power curves for the D20 data.

2. *Recessive Models* (*R20 and R80*)

When the GM is R20, the ELOD (table 3) for the R50 analysis is 10.1, versus 9.9 for MMLS-C, whereas the ELOD for the TRUE statistic is 10.2. When the GM is R80, the ELOD is 14.5 for MMLS-C, versus 15.6 for the TRUE statistic. Figure 2 shows the corresponding power curves for the data generated under the R20 model. Here, the situation is even more robust than when the GM is dominant. For the *Z* thresholds that we examined, there is little chance of missing a linkage if one uses MMLS-C (table 4), and the MMLS-C:TRUE ratio is quite close to 1.0, for all thresholds examined, for both R20 and R80 GMs.

Note, in figure 2, that the power for TRUE actually drops below the power for the R50 analysis, at high (i.e., >10) *Z* values. We investigated this phenomenon and discovered that, as the *Z* values increase, the data sets contain families with increasing numbers of affected members. For these data sets, the effective penetrance is actually higher than the 20% under which they were generated, and the R50 analysis provides a better description of the data than does the R20.

For the D20, D80, R20, and R80 models, the mean difference in ELODS between MMLS-C and TRUE is 1.08, and the range is 0.3–1.4. The mean percentage difference between MMLS-C and TRUE is 12%.

3. *Intermediate Models*

For the intermediate model with $f_2 = 10\%$, the ELOD for the TRUE statistic is 3.5, versus 2.8 for MMLS-C (table 3). As the penetrance rises and the model becomes more "dominant-like," the D50 analysis consistently outperforms the R50 analysis, and there is little drop in the ELOD for MMLS-C, compared with that for TRUE. Since f_2 is low, the analysis penetrance is quite different from the true penetrance. Despite the difference between the true and analysis penetrances, the ELODs differ by only 12%: 10.7 for the TRUE (Int80) analysis, versus 9.4 for the MMLS-C. There is even enough information at such a high penetrance to distinguish the models by

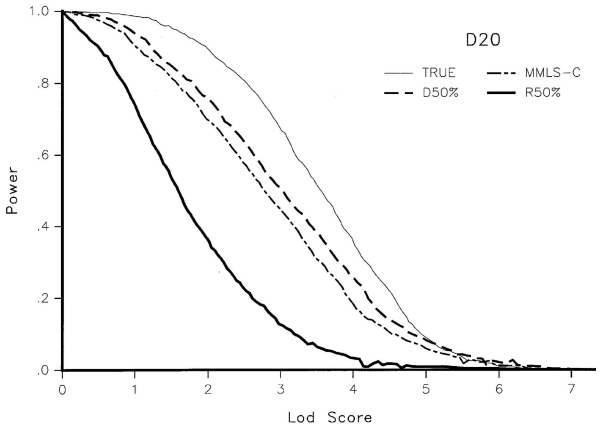

Figure 1 Power curves for D50, R50, MMLS-C, and TRUE analyses of 1,000 data sets generated under the D20 model.

Table 4

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

	Power to Achieve Z =											
	2.0			3.0			4.0			5.0		
Model	TRUE	MMLS-C	M:T Ratio	TRUE	MMLS-C	M:T Ratio	TRUE	MMLS-C	M:T Ratio	TRUE	MMLS-C	M:T Ratio
Simple Mendelian:												
D20	.89	.70	.79	.67	.45	.67	.36	.18	.50	.09	.06	.67
D80	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.99	.99	.99	.98	.99
R20	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
R80	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Intermediate:[a]												
.1	.89	.72	.81	.67	.42	.63	.34	.17	.50	.11	.06	.55
.3	.96	.87	.91	.82	.68	.83	.57	.42	.74	.29	.21	.72
.5	1.0	.99	.99	.96	.95	.99	.87	.83	.95	.73	.67	.92
.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.99	.99	1.0
Additive3[b]												
.01	1.0	1.0	1.0	.98	.98	1.0	.92	.92	1.0	.82	.77	.94
.05	.95	.90	.95	.80	.70	.88	.58	.43	.74	.30	.20	.67
.10	.96	.90	.94	.82	.72	.88	.57	.46	.81	.29	.23	.79
Additive2:[b]												
.01	.97	.93	.96	.87	.79	.91	.71	.57	.80	.51	.36	.71
.05	.41	.28	.68	.14	.09	.64	.04	.04	1.0	.01	.01	1.0
.10	.05	.04	.80	.00	.01	...	.0	.0	...	.0	.0	...

[a] Values shown (i.e., .1, .3 .5, and .8) are f_2; f_1 is fixed at .9.

[b] Values shown (i.e., .01, .05, and .10) are gene frequency at the unlinked locus.

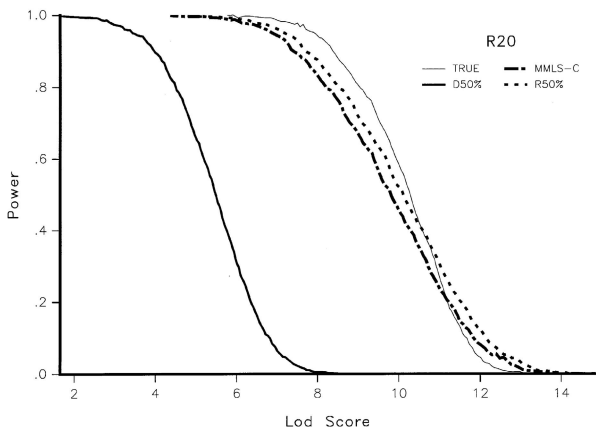

Figure 2 Power curves for D50, R50, MMLS-C, and TRUE analyses of 1,000 data sets generated under the R20 model.

maximization of f_1 and f_2 separately. In a model in which the penetrance is high, finding linkage is not generally a problem.

Figure 3 shows the power curves for one of the intermediate GMs (i.e., Int30). In the worst case, the MMLS-C:TRUE power ratio is 0.50; this occurs when $f_2 = 10\%$, for a threshold of 3.0 (see table 4). However, note that this is a situation with low power for *both* test statistics. For all cases in which the power of TRUE is $\geqslant 50\%$, the MMLS-C:TRUE ratio is not <.63.

4. *Additive Two-Locus Models*

a. "Additive3": three disease alleles required for disease expression.—These are probably the GMs that are most unlike either simple Mendelian model used to analyze them. When the disease-allele frequency at the unlinked locus is .01, the same as at the linked locus—that is, the gene-frequency combination is (.01,.01)—the ELOD is 6.5 for MMLS-C, versus 6.8 for TRUE. Examination of figure 4 suggests that recessive inheritance provides a good description for this model, at the gene frequencies used (i.e., .01,.01). For the gene-frequency combination (.01,.10), the MMLS-C ELOD is 3.9, whereas under the true model it is 4.3.

Figures 4 and 5 show power curves for these two GMs. Notice that an especially interesting transformation occurs in this model when the disease-gene frequency at the *unlinked* locus becomes 10-fold higher than that at the linked locus: When the disease-allele frequencies at the two disease loci are equal (each .01), the R50 analysis yields higher Z values than does the D50 analysis (fig. 4). However, when the disease-allele frequency at the unlinked locus is .10, the highest Z values occur under the D50 analysis (fig. 5). In between, when the gene frequency at the unlinked locus is .05, the dominant and recessive analyses yield results that are close together (graph not shown, but see table 4). In the worst case, the power of MMLS-C is only 67% of the power of TRUE. Again, this occurs when power is low for both statistics. For all cases in which the power of TRUE is $\geqslant 50\%$, the MMLS-C:TRUE ratio is never <.74 (table 4).

b. "Additive2": two disease alleles required for disease expression.—When two disease alleles are necessary, the power to detect linkage decreases as the frequency of the disease allele at the unlinked locus increases. This is not surprising, since, as the allele frequency at the unlinked locus increases, more people are affected because of the action of that locus alone. As the information for linkage decreases, so does the effect that the model assumptions have on the analysis. However, when the gene frequencies at the two loci are equal, using MMLS-C leads to an ELOD that is 87% of the value for the TRUE analysis—an ELOD of 4.5 for MMLS-C, versus an ELOD of 5.2 for TRUE. For these additive2 models, the worst MMLS-C:TRUE power ratio in our simulations was .64, but when the TRUE power was $\geqslant 50\%$ the MMLS-C:TRUE ratio was never <.71 (table 4). Figure 6 shows the power curves for one of these models, the additive2 GM with an unlinked-gene frequency of .1. Clearly, the power to detect linkage at all, under any analysis conditions, is relatively low, for this particular model.

Discussion

We undertook this simulation study to answer two questions. First, how much does using "corrected MMLS" (MMLS-C) decrease the power to detect linkage, compared with the "gold standard" of analyzing under the true model? Second, how well does MMLS perform, compared with the TRUE analysis, when the MOI of the disease is not simple Mendelian but the assumptions of the AM are simple Mendelian? We have

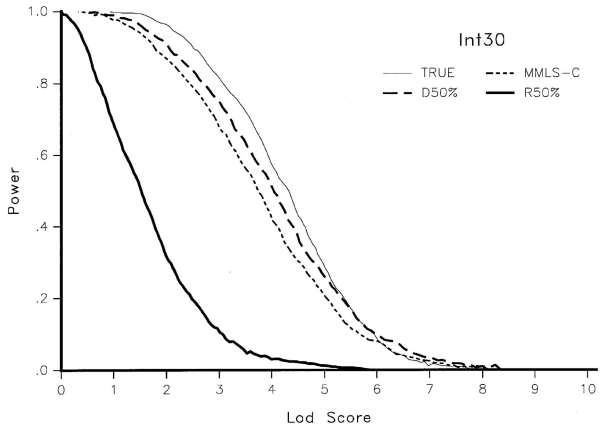

Figure 3 Power curves for D50, R50, MMLS-C, and TRUE analyses of 1,000 data sets generated under the Int30 model.

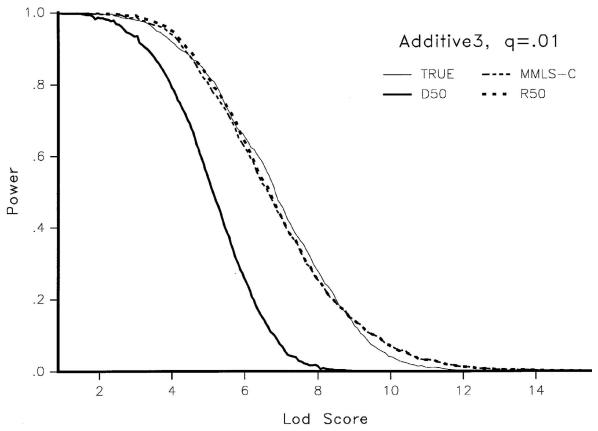

Figure 4 Power curves for D50, R50, MMLS-C, and TRUE analyses of 1,000 datasets generated under the additive3 model. The unlinked-gene frequency is .01. Note that the R50 model shows more power than does the D50 model.

demonstrated, within the limitations of our simulation models, that the MMLS-C approach does not substantially decrease the power to detect linkage, compared with what one would find if one could use the true MOI, and that assuming a simple Mendelian model works quite well even when the true model is relatively complex. In addition, we also have provided a baseline for comparison of other linkage-analysis methods.

In brief, for most of the 14 very different GMs that we considered, the differences, in ELODs (table 3), between the TRUE and MMLS-C analyses ranged between ~.3 (the lower bound; see eq. [1]) and .7. In the three cases in which this difference was >1.0 (GMs D80, R80, and Int80), the absolute ELODs were so high (all >9.0) that power was close to 1.0 for all thresholds examined (table 4). That is, the relatively larger difference in ELODs does not translate into practical concern. When we look at actual power levels for the four thresholds that we examined, we see that the MMLS-C:TRUE power ratio was very high (.99–1.00) when the TRUE power was very high (.99–1.00). When the TRUE power was $\geqslant$.75, the MMLS-C:TRUE ratio ranged from .79 upward. When the TRUE power was .50–.75, the MMLS-C:TRUE ratio dropped as low as .63 for one case (Int10 at threshold of 3.0) and .67 for another (D20 at threshold of 3.0), but otherwise the MMLS-C:TRUE ratio was >.70. The only times when the MMLS-C:TRUE ratio fell below those values was when the absolute power of TRUE was also low, <.50.

Note that, by comparing the MMLS-C analysis with the *true* analysis, we are putting the MMLS approach in the worst possible light. The *Z* value from the TRUE analysis, or "gold standard," represents an upper limit, and presumably *no* other analysis can do better than that, on average. In fact, this "upper limit" cannot, in general, actually be achieved when we do not know the true complex MOI. That is, this gold standard is not generally achievable in actual practice, since, by definition in these complex diseases, we do not know the true MOI. We are in the process of evaluating the question of when and under what conditions other analysis methods may have linkage-detection power that is equal to—or greater than—that of MMLS-C.

We have demonstrated that this approach is robust and has linkage-detection power that is often close to that of the TRUE model. This is the case not only when the true model is dominant or recessive—including the case in which there is reduced penetrance—but also for several models more complex than the dominant or recessive (see below). The correction for multiple testing (i..e., .3) is usually small, compared with the difference between the ELODs for the dominant and recessive models, even when the GM is D20. If the GM is recessive, the difference in power between the dominant and recessive assumptions dwarfs the information lost by correction for multiple testing.

Although this also was true of the intermediate model, we note that, when the homozygote penetrance is high (90%) and the heterozygote penetrance is quite low (10%), there is very little difference between assuming dominant and assuming recessive inheritance. In fact, the power to detect linkage at all, even when one assumes the true model, is low. (In work in progress, we have noted that the *Z* drops dramatically when the homozygote penetrance is high compared with the heterozygote penetrance, even when the data are analyzed at the generating penetrance values.)

One especially interesting observation sheds light on what factors are important in a locus-by-locus linkage

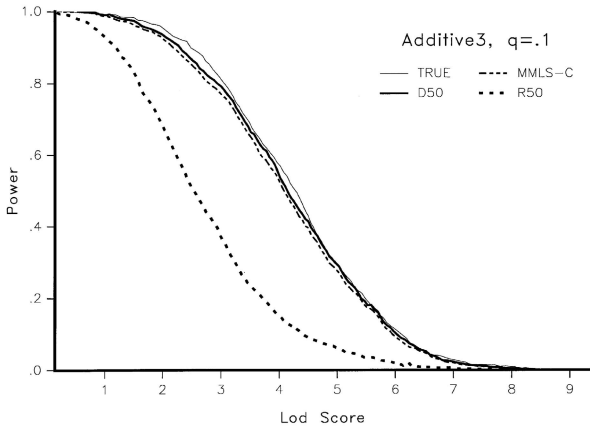

Figure 5 Data are as in figure 4, except that the unlinked-gene frequency is .1. In contrast to figure 4, the D50 model has more power than does the R50 model.

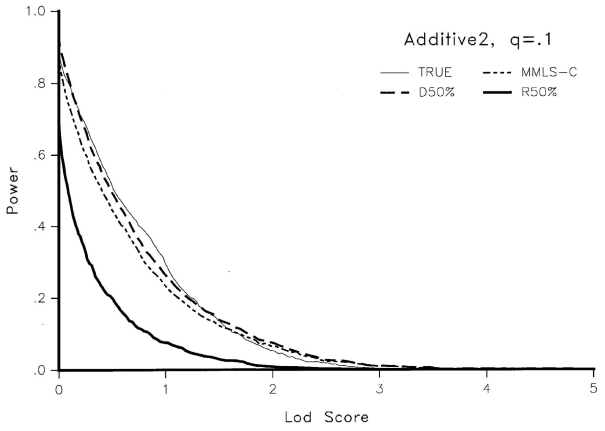

Figure 6 Power curves for D50, R50, MMLS-C, and TRUE analyses of 1,000 data sets generated under the additive2 model, when the unlinked-gene frequency is .1.

scan. In our results with the additive3 model, we found that the gene frequency *at the unlinked locus* determined which assumed MOI at the linked locus led to the higher *Z* value. The reason for this dependence is as follows: If the gene frequency at the unlinked locus is low, then it is more probable that all individuals will have one disease allele, rather than two, at that locus. If so, then there must be two disease alleles at the linked locus, in order for an individual to be affected. Similarly, if the gene frequency at the unlinked locus is high, then there is a higher probability that an affected individual will be homozygous for the disease allele at the unlinked locus, and then there need be only one allele at the linked locus, in order for the individual to manifest the disease. Thus, in an additive-type model such as we have examined, the apparent MOI at the locus linked to the marker can change in response to what is going on at other loci.

This last point has interesting implications for linkage analysis of complex diseases. In an additive model, the *apparent MOI* at a specific locus can change in response to changes in the gene frequency at other loci that contribute to the disease phenotype. The results of a segregation analysis in a situation such as this might not be very useful (also see below). In addition, the MOI for different populations could vary.

We also found it interesting that, for the additive3 model, when the true MOI was used to calculate the *Z* value, it led to a Z_{max} only slightly higher than the higher of the two *Z* values calculated under the two simple AMs (mean difference .55, or 8%). This was true despite the fact that the GMs were not simple Mendelian models. This also suggests that the dominant and recessive models, despite their simplicity, provide a reasonable approximation when we are using linkage analysis for a locus-by-locus search for disease genes. It is, in fact, tortuous to imagine a genetic model in which the inheritance at that locus would *not* approximate dominant or recessive inheritance, provided that the linked locus has a specific allele or alleles that affect the expression of the disease. The effect of the allele may be too small to be detected by linkage analysis or may be affected by other loci, but the allele still must be transmitted.

In some of the models that we examined—for example, the additive2 model with gene frequency .01 for the linked locus but .1 for the unlinked locus—linkage was simply difficult to detect. This difficulty was inherent in the GM, and this difficulty would also hold for *any* AM. For example, in the additive2 example just mentioned, the unlinked locus would have a greater influence on the disease than the linked locus does, because of its 10-fold–higher gene frequency. However, in a genome search the unlinked locus in this example would be more easily discovered.

The MMLS approach that we have tested here is simple and is predicated on the notion that, in linkage analysis, when it is done one locus at a time, the MOI *at the locus being tested*—not the inheritance of the disease per se—is the critical assumption. Thus, this study suggests that parameters derived from a segregation analysis should be applied with caution in a subsequent linkage analysis. Dizier et al. (1996) reported that, in certain complex models, the ASP method appeared to have more power to detect linkage than did *Z* values. However, Dizier et al. used parameters derived from a segregation analysis. As a result, although 11 of the models they investigated found that *Z* values had higher power to detect linkage than was seen in the ASP method, in three of the models the ASP method had higher power. Closer examination of the models used by Dizier et al. shows that, for those two models in which the *Z* method was weaker than the ASP approach, the MOI at the *linked* locus was recessive although the segregation analysis suggested dominant inheritance of the disease. Had Dizier et al. analyzed the data in the manner that we have advocated here—that is, by means of both a dominant and a recessive MOI—the *Z* method would have proved more powerful in *all* cases (Durner et al. 1997), thus undermining Dizier et al.'s conclusion that there are circumstances in which ASPs have more power to detect linkage than does *Z* analysis.

In previously published work, we have shown, via simulations, that, when one maximizes the *Z* over the dominance model, .3 represents a reasonable approximate correction factor; that is, one should decrease the observed maximized Z_{max} by .3, to allow for having performed multiple tests. This value of .3 represents a conservative value. Although the correction factor was de-

rived for dominant and recessive models, we know that there is no information about MOI if there is no linkage. Thus, in the absence of linkage, type I error is approximately the same no matter what MOI is assumed (Williamson and Amos 1990; Hodge and Elston 1994). Therefore, the MOI used to analyze the data should not affect the type I error or our cutoff threshold. Also, the accumulating evidence is that dominant and recessive models with reduced penetrance provide good approximations of the effect of other loci. This suggests that .3 will be a conservative correction factor for performance of two Z analyses, no matter what the true MOI, although we cannot yet prove that.

When we originally derived the correction factor for multiple testing (Hodge et al. 1997), we found that the two-sided χ^2 provided a reasonable approximation for derivation of the correction factor. In that work, our simulations only went up to Z_{max} values of 3.0. In the current work, we have much higher Z_{max} values, so we have calculated an approximate correction factor for higher Z_{max}, based on going from a one-sided to a two-sided α. For $Z_{max} \leqslant 13.9$, this approximate correction factor is .293, very close to the .284 found for a $Z_{max} = 3.0$. In any case, we used a correction factor of .3, which is slightly more conservative.

If one also maximized over penetrance, an additional .3 Z unit (conservatively) would need to be added to the correction factor. However, in the current work, we fixed the assumed penetrance at an arbitrary 50%, so that we needed to correct only for the dominance-model maximization. We did not maximize over penetrance, because we wanted to see whether our approach led to good power to detect linkage while keeping the number of separate analyses to a minimum (i.e., two). Our findings again confirmed that the wrong penetrance assumptions alone had relatively small effects on the magnitude of the Z value (Clerget-Darpoux et al. 1986; Greenberg 1989). However, there are situations (e.g., the D20 and R20 models) in which maximization with respect to penetrance would increase power more than the power loss due to type I error. As we have noted above, for most GMs that we examined, maximizing the penetrance would not have increased the ELODs by more than would have been lost by additional correction for multiple testing.

We used two-point analyses for these calculations, but, in work in preparation, the results also apply equally to multipoint analysis. We chose to use two-point analyses because they require significantly less computation time and because the conclusions for multipoint analysis are identical to those for two-point analyses, in all cases that we have examined so far. Also, two-point analyses are often best suited for initial genome screens.

In the absence of any knowledge about the inheritance of the disease at a locus, one could either make assumptions about the MOI, in order to carry out a Z analysis, or use "nonparametric" affecteds-only methods, which may not be as powerful and do not use all the available data (Hodge 1998). As demonstrated by Knapp et al. (1994) and Whittemore (1996), although the affecteds-only methods may be free of explicit mode-of-inheritance assumptions, the characteristics of the tests have statistical properties that are similar to the properties of maximum-likelihood tests *under specific genetic models*. For example, Knapp et al. demonstrated that one ASP test, the Mean Test, has statistical properties *identical* to those of a Z analysis assuming recessive inheritance.

Like all simulation-based studies, this one looked at some specific models and situations. Even though we confined the GMs to one-locus and two-locus models, the range of models was large. Even more important, the pattern that emerged, first observed with the work on two-locus epistatic models (Greenberg and Hodge 1989; Vieland et al. 1992), is that it is the MOI at the linked trait locus that is important in a genome search. At that locus, either one or both alleles contribute to trait expression, making the assumption of dominant or recessive inheritance quite robust for linkage detection. Also, of course, the absolute power levels and ELODs will vary with sample size and other factors. However the *relative* power levels and ELODs reported here should have wide application.

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References

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

Clerget-Darpoux F, Bonaïti-Pellié C (1992) Strategies on marker information for the study of human diseases. Ann Hum Genet 56:145–153

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

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

Durner M, Vieland VJ, Greenberg DA (1997) Increased power of lod scores over ASP methods. Am J Hum Genet Suppl 61:A274

Elston RC (1989) Man bites dog? the validity of maximizing

lod scores to determine mode of inheritance. Am J Med Genet 34:487–488

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

Greenberg DA, Hodge SE, Vieland VJ, Spence MA (1996) Affecteds-only linkage methods are not a panacea. Am J Hum Genet 58:892–895

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

Haseman JK, Elston RC (1972) The investigation of linkage between a quantitative trait and a marker locus. Behav Genet 2:3–19

Hodge SE (1998) Exact ELODs and exact power for affected sib pairs analyzed for linkage under simple right and wrong models. Am J Med Genet (Neuropsychiatr Genet) 81:66–72

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

Knapp M, Seuchter SA, Baur MP (1994) Linkage analysis in nuclear families. 2. Relationship between affected sib-pair tests and lod score analysis. Hum Hered 44:44–51

Kruglyak L, Lander ES (1995) High-resolution genetic mapping of complex traits. Am J Hum Genet 56:1212–1223

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

Leder RO, Mansbridge JN, Hallmayer J, Hodge SE (1998) Familial psoriasis and HLA-B: unambiguous support for linkage in 97 published families. Hum Hered 48:198–211

Ott J (1974) Estimation of the recombination fraction in human pedigrees: efficient computation of the likelihood for human linkage studies. Am J Hum Genet 26:588–597

Suarez BK, Van Eerdewegh P (1984) A comparison of three affected-sib-pair scoring methods to detect HLA-linked disease susceptibility genes. Am J Med Genet 18:135–146

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

Weeks DE, Lange K (1988) The affected-pedigree-member method of linkage analysis. Am J Hum Genet 42:315–326

Whittemore AS (1996) Genome scanning for linkage: an overview. Am J Hum Genet 59:704–716

Williamson JA, Amos CI (1990) On the asymptotic behavior of the estimate of the recombination fraction under the null hypothesis of no linkage when the model is misspecified. Genet Epidemiol 7:309–318