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HLA and the Inheritance of Multiple Sclerosis: Linkage Analysis of 72 Pedigrees

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SUMMARY

Linkage analysis of 72 pedigrees by the maximum-likelihood method provides evidence of linkage between *HLA* and the hypothesized multiple sclerosis susceptibility gene (*MSSG*) for both the dominant and recessive models of inheritance and for penetrance values ranging from 5%–65% (or higher). This *MSSG*, if it exists, is most likely located at 15%–20% recombination units from the *HLA* complex, probably on the *B-D* side. The analysis also shows that there is no heterogeneity in the estimates of linkage, and lod scores are not artificially inflated because of the association of multiple sclerosis (MS) with HLA-B7.

INTRODUCTION

Multiple sclerosis does not exhibit a clear-cut Mendelian segregation pattern in families, and, hence, the genetic hypotheses of both autosomal dominant genes and recessive genes with reduced penetrance and multifactorial determination have been proposed. Although Myrianthopoulos and MacKay reported that the observed segregation pattern shows recessive inheritance with a penetrance value of 43.2% [1–3], McAlpine [4] and Whelan and Poskanzer [5] argued that with the concept of reduced penetrance, any genetic hypothesis can be used to fit the data (for an excellent exposition, see Edwards [6]). Visscher et al. [7], however, postulated dominant transmission with incomplete penetrance of 5%. In this study, we examine both dominant and recessive models.

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An association of MS with an HLA antigen was first shown, by us, in 1972 [8]. Since, more than 15 studies have investigated the association at the population level and have led to the hypothesis of an *MSSG* on chromosome 6. A comprehensive analytical review of this subject has been published by Jersild [9].

Ten recent published reports on the segregation of MS using *HLA* as a genetic marker have shown both variable and inconsistent results. Some family studies [10–14] showed MS segregating with the marker haplotypes, thus indicating the presence of an *MSSG* in close linkage with the *HLA* loci. Others [15–17] report the segregation of *MSSG* independent of the *HLA* loci (i.e., no distortion in the segregation ratios) and have examined the joint segregation of *MS* and *HLA* haplotypes in sibs by comparing observed and expected segregation ratios.

This study pools all the data of these reports and utilizes more effectively information from all members of the families —in particular, generations generally not considered. Furthermore, none of these reports performed a formal linkage analysis. Alter et al. [12] computed lod scores but did not include reduced penetrance in his calculations. We also examine whether the association is artificially inflating the linkage results and test for heterogeneity. This report provides a complete and mathematically correct linkage analysis of MS and HLA in a large body of family data.

DATABASE AND METHODS

The authors of the 10 reports, from which a total of 72 pedigrees was obtained, together with MS diagnosis criteria, are given in table 1. About one-third of these pedigrees were HLA-typed in our laboratory.

The estimates of recombination were obtained by the standard maximum-likelihood method [21–23], indicating which recombination fraction value is "most likely" to have produced the families observed. The lod scores were computed using Ott's LIPED program [24]. We assumed that the recombination fraction was the same in both sexes. We also used only *A-B* haplotype data, even though stronger MS associations have been reported with the *D* locus. Since these loci are closely linked, the results should be approximately the same.

TABLE 1

SOURCE, DIAGNOSTIC CRITERIA, AND HETEROGENEITY OF LINKAGE ESTIMATES OF PEDIGREES USED IN LINKAGE ANALYSIS

Study	Study code	No. pedigrees	MS diagnosis criteria	df	Dominant *MSSG* with 5% penetrance	Recessive *MSSG* with 43% penetrance
Alter et al.	A	10	Schumacher et al. [18]	9	5.554	9.159
Bertrams and Kuwert	BK	11	...	10	4.163	9.141
Bird	B	1*	...	...	...	...
Drachman et al.	D	8	Schumacher et al.	7	5.673	9.150
Eldridge et al.	E	7*	Schumacher et al.	6	2.676	3.937
Hens and Carton	H	13	Schumacher et al.	12	5.660	9.477
Jersild et al.	J	1	Broman et al.	...	...	...
Olsson et al.	O	8	Schumacher et al.	7	2.920	2.095
Trouillas and Betuel [19]	T	1	Broman et al. [20]	...	...	...
Visscher et al.	V	12*	Schumacher et al.	11	0.005	5.162
Total		72		71	39.939	68.532

* Families typed in our laboratory (P. I. Terasaki).

Coding and Specification of the Marker Locus

In coding the marker alleles, all information was copied exactly as published, with obvious misprint errors corrected. The *HLA* allele and haplotype frequencies were taken from *Histocompatibility Testing, 1977* [25].

All data were analyzed twice: first, using the *HLA-A* and *-B* loci as two independent markers and, second, using the *A-B* haplotype as a single marker. The first method is useful for fine mapping and also serves as a control, as in detecting errors during coding and keypunching. However, the haplotype method reflects the genetics of the *HLA* system more accurately and uses computer time more efficiently [26]. Our results are based on the haplotype method of scoring. Recombinant individuals cannot be included in a haplotype analysis and must, therefore, be omitted. In our data, there are three pedigrees with such individuals. Two of the persons (in Olsson et al., family B [14] and Visscher et al., family D [7]) did not have the disease, and deleting them did not noticeably affect the families' lod scores. In the third family (Alter et al., family K [11]), the recombinant individual was affected with MS. This family was analyzed both by the haplotype method, omitting the recombinant person, and by using separate *A* and *B* loci, with that person included.

Specification of the MSSG Locus

All individuals classified as "possible MS," "probable MS," and "definite MS" were specified as affected. In the linkage analysis, the mode of inheritance of MS must be specified. Without clear evidence for a dominant or recessive mode of inheritance, all the pedigree data were analyzed twice using these two genetic models. Model choice, in turn, affects the gene frequency of the *MSSG* allele.

Under both models, let T be the dominant gene, with frequency p, and t, the recessive allele, with frequency $q = 1 - p$. Define the penetrance f as that fraction of all individuals possessing the at-risk genotype who are, in fact, affected with MS. Also note that in high-risk areas, the prevalence of MS is 20–90/100,000 [27, 28]. We will use a prevalence of .0005 as an average value. (Lod scores are not sensitive to fluctuations in disease prevalence or gene frequency [24].) We also assume Hardy-Weinberg equilibrium.

Thus, under a dominant model with incomplete penetrance, in which both *TT* and *Tt* genotypes are at risk, the prevalence of MS is given by

$$(1 - q^2)f = .0005 \tag{1}$$

By assuming the lifetime probability of developing MS in a high-prevalence area to be .001 and taking a 25-fold increase of prevalence in first-degree relatives, Visscher et al. [7] estimated the penetrance to be 5%. Substituting this in equation (1) yields the *MS* gene frequency $p = .005$.

Similarly, under a recessive model with incomplete penetrance, in which only *tt* individuals are at risk for the disease, the prevalence of MS is given by

$$q^2f = .0005 \ . \tag{2}$$

Myrianthopoulos [3] suggested a recessive mode of inheritance with a pentrance value of 43%. Substituting this value of f in equation (2), we have $q = .034$.

RESULTS

Linkage Analysis

The lod scores for the 72 families were calculated using the *HLA-A-B* haplotype as the marker. Two genetic models for *MSSG* inheritance were examined: dominant inheritance with 5% penetrance and recessive inheritance with 43% penetrance. The results are given in figures 1 and 2.

In the dominant model, the maximum score was 2.786 at $\theta = .15$, giving odds of

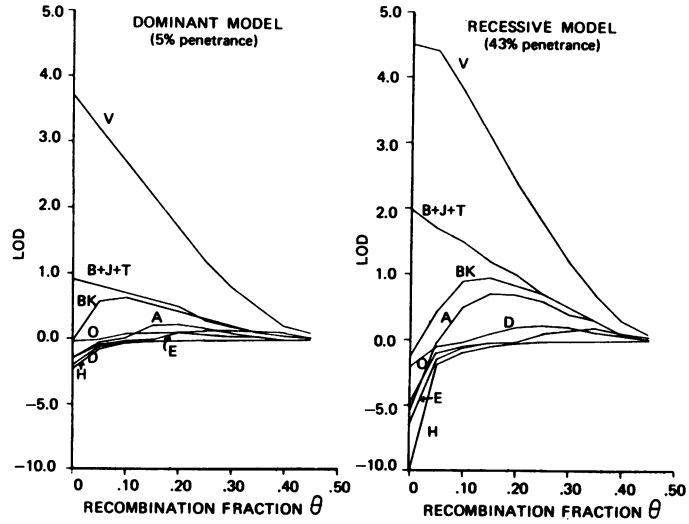

FIG. 1. —Total lod scores for each study. *Letters* correspond to studies in table 1

611:1 ($P \sim .002$) in favor of linkage (fig. 2). These data provide highly suggestive evidence of linkage between an *MSSG* and *HLA*.

The maximum lod score for the recessive model was 4.089 at $\theta = .20$ with odds of 12,274:1 ($P = 8.2 \times 10^{-5}$) in favor of linkage (fig. 2). This is substantially higher than the odds in the dominant model with 5% penetrance.

Both models provide similar results within any given study; however, there are two

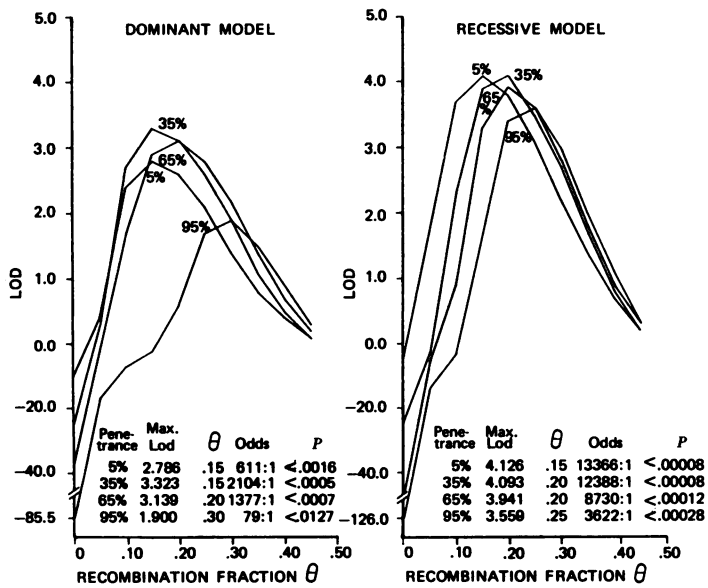

FIG. 2. —Effects of penetrance parameter on estimates of recombination fraction

noticeable differences between the two models. First, in the recessive model, except for the families of Drachman et al. [15] and Olsson et al. [14], the total lod scores for each study are slightly higher, resulting in the overall maximum of 4.089 at $\theta = .20$. Second, the total scores from these families have changed from negative to positive values and from positive to negative, respectively. This probably reflects the inconsistency in the segregation of MS in some of the families.

The most interesting results are from the 12 families of Visscher et al. [7]. All but one family yielded relatively large positive scores. The maximum for these families was 3.725 at $\theta = .00$, indicating very close linkage. Based on all 72 families, however, we do not have evidence for very close linkage (see fig. 1).

When the recombinant daughter (affected by MS) in family K of Alter et al. [12] was deleted from the analysis, the family's scores were positive, although negligible for all values of θ (e.g., lod = .01 at $\theta = .0$). When *HLA-A* and *-B* were used as two independent markers and the recombinant daughter was included, the scores were all negative with respect to the *A* locus (because of the recombinantion between the *A* and *B* loci) and positive with respect to the *B* locus. The maximum was .305 at $\theta = 0$. Thus, although lod scores from the two methods of coding markers cannot legitimately be combined, this family provides evidence that the *MS* susceptibility locus is located on the "*B*" side of the *HLA* complex, so that in the recombination between *A* and *B*, it segregates with the *B* locus. This agrees with the conclusions from the association data from the population studies.

We examined the effects of varying the penetrance parameter on the linkage analysis. The results for penetrance values of 5%, 35%, 65%, and 95% and the odds and probabilities associated with these values are given in figure 2. These results show strong evidence of linkage even at the 95% penetrance level, although the linkage is looser at higher penetrance values. The epidemiology of MS [27, 28] shows environmental factors play a significant role in its etiology, and thus the value of the penetrance parameter cannot be very high. The evidence for linkage is much stronger at the lower values of penetrance.

Heterogeneity of the Linkage Estimates

There is variability among individual pedigrees as well as among studies. For example, in the studies of Drachman et al. [15], families B, O, Q, and V showed negative scores and families H, L, S, and Br showed positive scores. This variability may be due to random effects or to actual heterogeneity between the two groups exhibiting positive and negative lod scores. Heterogeneity could exist with respect to age, sex, genetic background, environment, chromosomal rearrangements, or two different forms of the disease, such as Morton [29] found in the case of elliptocytosis and the Rhesus blood group.

To examine the heterogeneity of the linkage estimates, we utilized the test suggested by Morton [29] (also see Smith [30]). If $\hat{z}_i$ is the maximum lod score of the ith pedigree and $\hat{Z}$ is the maximum lod score from all the pedigrees, then it can be shown that the quantity

$$\alpha = \left(\sum_{i}^{n} \hat{z}_i - \hat{Z}\right) \times 4.605$$

is approximately distributed as χ^2 with $n - 1$ degrees of freedom; n is the number of pedigrees in the sample. Results of this test for the dominant and recessive models with 5% and 43% penetrances, respectively, are given in table 1. The χ^2 values are not significant at the 5% level. Thus, although there is some variability between the pedigrees and also between the studies, it does not produce significant evidence of heterogeneity, which is consistent with the hypothesis that MS is a single disease. The results of the heterogeneity tests for other penetrance parameter values considered in this analysis were similar to those given in table 1.

Effects of the Association of MS with B7

Since MS is associated with HLA-B7, the calculated total lod scores may be wholly or partly inflated. If the *MSSG* is linked with *HLA*, then the association with B7 presumably reflects linkage disequilibrium between the two loci, as opposed to a causal association.

We know of no formal mathematical method for separating the effects of the association from the total lod score. We divided the data set into 43 families with the *B7* allele and 29 families without it. Figure 3 shows the lod scores broken down into these two sets for the dominant and recessive models, respectively. In the dominant model, the 43 families with B7 had a maximum score of .6 at $\theta = .20$, and the remaining 29 families had a maximum of 2.3 at $\theta = .15$. Surprisingly, the non-B7 pedigrees contribution is much greater than that of the B7 pedigrees. All the pedigrees do not contribute equal information toward linkage and, hence, cannot be assigned equal weight. Also, one particular family, 01z [12], contributed disproportionately to the negative total lod score of the B7 families.

Nonetheless, the families with B7 constituted 60% of the total sample, yet contributed less than 22% toward the maximum score of 2.8. In fact, we could ignore

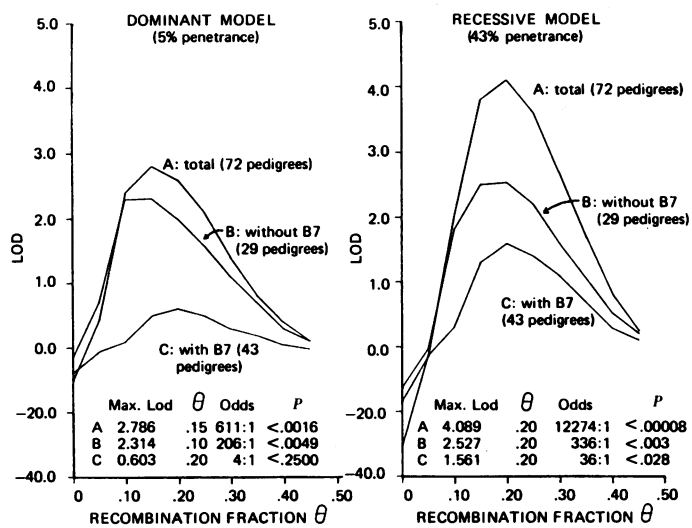

FIG. 3. —Total lod scores for B7, non-B7, and B7+non-B7 pedigrees

all the families with B7 and still have some evidence for linkage. Thus, we conclude that the total lod scores were not artificially inflated by the association of MS with B7. Opposite results in figure 3, that is, high scores contributed by the B7 group, would cast serious doubt on the existence of true linkage. As figure 3 shows, the B7 group's contribution in the recessive model was also much less than that of the non-B7 group.

DISCUSSION

Our analysis of the 72 pedigrees shows evidence of linkage between *HLA* and the hypothesized *MSSG* with penetrance values ranging from 5% to almost 100% in both dominant and recessive modes of inheritance. In addition, the test gives no evidence of significant heterogeneity of the recombination estimates, and the separation of the lod scores into the categories of B7 and non-B7 pedigrees indicates that the total lod scores are not inflated by the *B7* allele-*MSSG* association. These results provide additional support for the existence of true linkage between *HLA* and the hypothesized *MSSG*.

The penetrance parameter and the mode of inheritance of MS, however, influence the recombination estimate. In the dominant model, for the penetrance values of 5%, 35%, 65%, and 95%, the maximum lod scores were obtained at θ equal to .15, .15, .20, and .30, respectively. The recessive model had maximum lod scores at θ equal to .15, .20, .20, and .25 for the same respective penetrance values (fig. 2). Thus, it seems that the distance between *HLA* and the *MSSG* is somewhere in the range of 15% – 30% recombination, the actual value dependent upon the mode of inheritance and the penetrance parameter. If the stronger association of MS with the *HLA-D* locus is due to stronger linkage disequilibrium, then the disease gene is located on the *B-D* side of the *HLA* complex, as confirmed by family K of Alter et al. [12].

Since environmental factors play a large role in the etiology of MS, the penetrance value cannot be very high. If we assume that 95% penetrance is unlikely, then the estimates of the distance fall within a narrower interval of 15% – 20% recombination. Thus, for penetrance values ranging from 5% – 65% (or higher), both dominant and recessive models give very similar recombination estimates. At very low penetrance values, these estimates are identical (fig. 2). For all penetrance values in both models, evidence against very close linkage (less than 5% recombination) is exceedingly strong.

Our results are based on the assumption of single-gene (i. e., dominant or recessive) inheritance with reduced penetrance; however, the accuracy of this assumption has not been established. That the evidence for linkage is present regardless of the model used raises some questions about the mode of inheritance of MS. Perhaps, as has been suggested recently for juvenile insulin-dependent diabetes [31], MS results from epistatic interactions between two loci, only one of them located in or near the *HLA* complex. Such a situation could mimic loose linkage.

It is important that these results be verified by data on other genetic markers on chromosome 6. Fortunately, the following three other well-known markers could provide such data.

HLA-D locus. Information about this marker is not available in most of the pedigrees in this analysis. Since the distance between the *HLA-D* and the *HLA-B* locus is less than 1% recombination, the recombination estimate between the *D* locus and the *MSSG*

should be very similar to the one observed in this analysis (using *HLA-A-B* haplotypes).

Phosphoglucomutase (PGM_3) and glyoxalase 1 (GLO) loci. The distance between *HLA* and PGM_3 is 20% and that between *HLA* and *GLO* is 10% recombination [32]. These two loci are located on the *B-D* side of the complex. If the distance between the *MSSG* and *HLA* is 15% – 20% recombination (on the *B-D* side), then the *MSSG* locus should be in much closer linkage with the PGM_3 and *GLO* loci. Thus, these three markers would provide valuable data to both verify and precisely locate the *MSSG* on the short arm of chromosome 6.

Recently, the gene for spinocerebellar ataxia has been mapped on chromosome 6 at a distance of about 22% recombination from *HLA* [33 – 35]. If this locus is also on the *B-D* side of the complex, then it must be very close to the *MSSG* locus.

The association of HLA-B7 with MS in random patients also poses a paradox. The association itself was the principal reason for suspecting the linkage, yet, among the families with HLA-B7, evidence of linkage was lower than among families without HLA-B7. However, our finding of loose linkage is not in itself at variance with the HLA-MS population associations. Linkage disequilibrium (rather than tight linkage), a possible cause of the association, may be due to selection favoring certain haplotypes.

In conclusion, by performing a complete and mathematically accurate lod score analysis of linkage, we have shown that if an *MSSG* with reduced penetrance exists, which is transmitted either dominantly or recessively, then this gene is very likely located on the short arm of chromosome 6, about 15 – 20 recombination units from the *HLA* complex, and probably on the *B-D* side toward the centromere.

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