# Analysis of Linkage between the Major Histocompatibility System and Juvenile, Insulin-Dependent Diabetes in Multiplex Families

## REANALYSIS OF DATA

JOSE BARBOSA, M. MYRA CHERN, HARRIET NOREEN, and V. ELVING ANDERSON, *Section of Endocrinology, The Department of Medicine, and The Departments of Pathology and Laboratory Medicine, and The Dight Institute for Human Genetics, University of Minnesota, Minneapolis, Minnesota 55455*

EDMOND J. YUNIS, *Department of Pathology, Harvard University Medical School, Boston, Massachusetts 02115*

ABSTRACT Linkage analysis between the major histocompatibility system (HLA) and juvenile, insulin-dependent diabetes, assuming an autosomal recessive mode and 50% penetrance was performed on 21 juvenile, insulin-dependent diabetic multiplex families (two or more diabetics per sibship) with phenotypically normal parents. The total lod score was the highest (3.98) at a recombination fraction of 13%. For a penetrance of 100%, the highest total lod score was 2.92 at a recombination fraction of 18%. These results are compatible with the existence of linkage between an autosomal recessive diabetic gene with 50% penetrance and the HLA in some of the families studied.

Our ascertainment strategy would be expected to increase the likelihood of selecting for genetically homogenous diabetes and against sporadic forms of the disease. Thus, our findings may apply only to a small proportion of all cases of juvenile, insulin-dependent diabetes.

## INTRODUCTION

We have recently reported a study of histocompatibility (HLA)[1] haplotypes (A and B alleles) on 24 diabetic multiplex families (1). These families were ascertained for the existence of two or more juvenile, insulin-dependent diabetic (JIDD) children in each sibship. This ascertainment strategy was aimed at obtaining for study a genetically homogenous type of JIDD. 55% of the diabetics in these sibships were HLA identical (i.e. shared both maternal and paternal haplotypes) as opposed to the expected 25% HLA identity.

This abnormal HLA haplotype assortment suggested that there may be one or more genes with a role in the pathogenesis of JIDD and in close association with the HLA system. The fact that in 21 of the reported 24 families, both parents are phenotypically normal is compatible with a double-dose gene mechanism for the hypothetical diabetic gene. In this paper we report on linkage analysis studies on the 21 families with normal parents and two or more affected children in the same sibship.

## METHODS

The clinical material in 24 diabetic multiplex families and the immunological studies of the HLA have been published (1). Only the 21 families with affected members in the second generation are included in this work. Thus, family A. P., with affected members in the third generation, and families S. Ros. and M. R. with affected parents (1) were excluded. In the latter family the mother became an insulin-dependent, ketosis-prone diabetic since the publication of our previous report. Furthermore, in this family, JIDD is associated with Hashimoto's thyroiditis and Addison's disease unlike any of the other families. Therefore, this may represent a different type of diabetes.

The linkage analysis was performed with the aid of the computer program LIPED (2), using the lod score method. This method is most often used to measure linkage in human pedigrees. It consists in comparing the likelihood of obtaining the pedigree on the assumption of several recombination values between two loci as compared with a recombination value of 50%, i.e., no linkage. Data from different pedigrees

*Received for publication 5 May 1978 and in revised form 30 May 1978.*

[1] *Abbreviations used in this paper*: HLA, major histocompatibility system; JIDD, juvenile, insulin-dependent diabetes mellitus.

can be combined by adding the logs of the probabilities (log odds or lod). The recombination value with the maximum lod score is the most probable value of the distance between the loci. The autosomal recessive mode of inheritance was assumed for JIDD. The level of penetrance was varied from 100 to 50% at a 10% interval.

The results of the linkage analysis were tested for heterogeneity by published methods (3).

## RESULTS

Fig. 1 shows the total lod scores for linkage between the HLA and JIDD in the 21 diabetic multiplex families assuming an autosomal recessive mode and penetrance values of 100, 90, 70, and 50%. For higher values of the recombination fraction (30 and 40%), the lod scores remain stable at the various levels of penetrance assumed. For the recombination fractions below 20% there is an inverse relationship between the lod scores and the penetrance assumed, i.e., the lower the penetrance the higher the lod score. This trend is evident at a recombination fraction of 13%, where the lod score for 50% penetrance is 3.98 and for 100% penetrance is 2.47. The highest lod score at 100% penetrance is 2.92 at a recombination fraction of 18%.

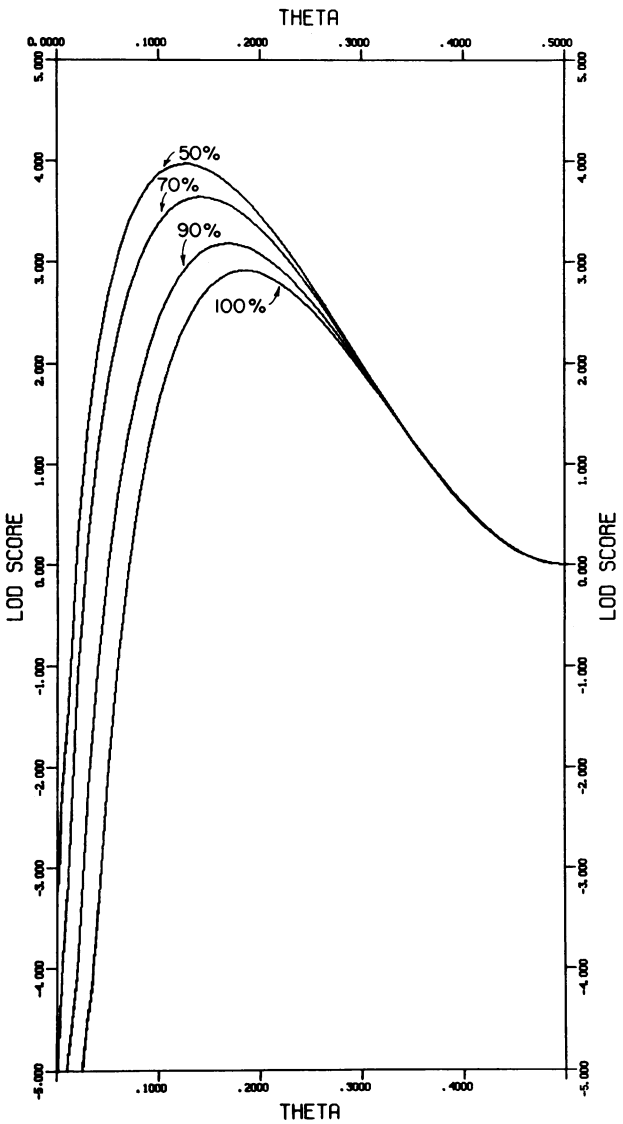


FIGURE 1 Total lod scores for linkage between diabetes and the HLA in 21 diabetic multiplex families assuming the autosomal recessive mode and penetrances of 100, 90, 70, and 50%. The total lod score is highest for 50% penetrance. THETA-recombination fraction.

Fig. 2 depicts the individual family lod scores at 50% penetrance assuming the autosomal recessive mode. By inspection there seems to be two groups of families, one of which (S. Rus, A. S., L. E., B. P., S. C., E. H., and P. F.) shows no linkage, i.e., negative or zero lod scores. A test for heterogeneity, however, does not rule out the null hypothesis of no linkage heterogeneity ($\chi^2$ = 13.88, 20 df). Lack of heterogeneity is also observed when assuming 100% penetrance ($\chi^2$ = 15.81).

The effects of reduced penetrance on the lod scores for the most likely recombination fraction of 13% are further analyzed in Fig. 3. The lod scores for each family assuming a penetrance of 50% were plotted against the lod scores for 100% penetrance. The majority of the families cluster along the 45° line, showing little change in the lod scores with the reduction in penetrance.

Families with children that are fully concordant for HLA type but discordant for JIDD (D. D., C. L., J. R., B. L., and P. D.) show a positive effect on the likelihood for linkage with reduced penetrance. They contribute the greatest increase for the total lod score (1.59) when changing from 100 to 50% penetrance. Family L. E. has a negative lod score at either penetrance level, owing to the fact that the two diabetic sibs share only one HLA haplotype. However, its lod score is improved by 0.19 as a result of allowing the two normal sibs fully HLA concordant with one or the other affected sibs some chance to have the presumed diabetic genotype in the reduced penetrance situation. Family H. H. shows the largest decrement in the lod score from 100 to 50% penetrance level, although with a positive score at both levels. This family has two affected sibs who are fully HLA concordant and three unaffected showing the three other possible haplotype combinations. The high likelihood of linkage shown by the fully HLA concordant siblings in the JIDD pair is reduced as the level of penetrance assumed is decreased.

## DISCUSSION

In our previous report on diabetic multiplex families (1), based on an analysis of the segregation of HLA

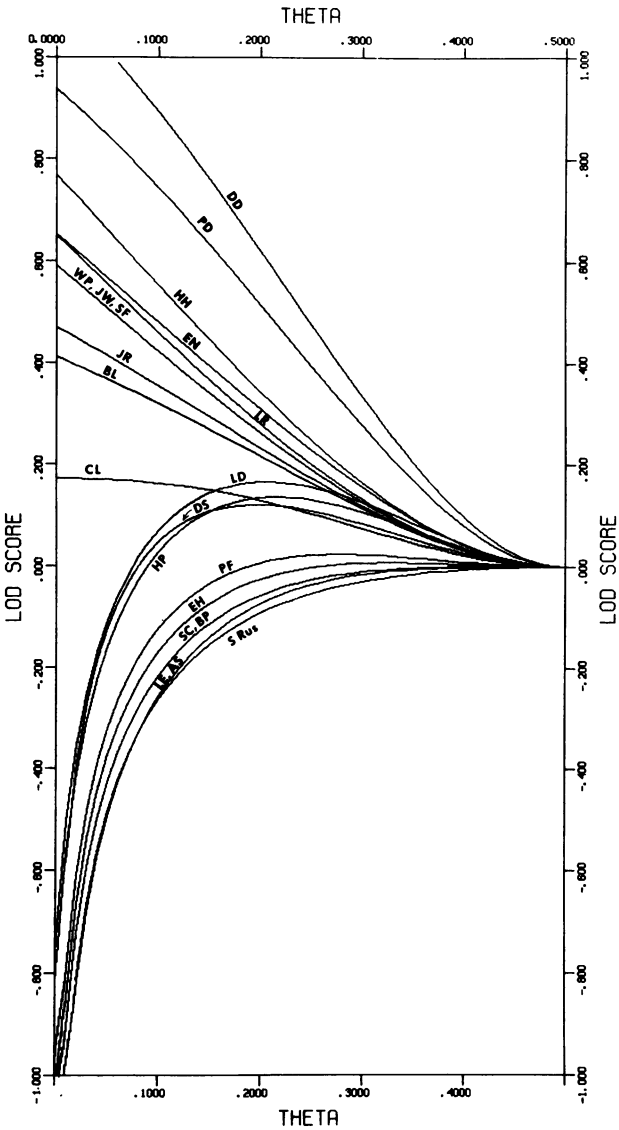


FIGURE 2 Lod scores for linkage between diabetes and the HLA in 21 diabetic multiplex families assuming the autosomal recessive mode and 50% penetrance. The highest total lod score is 3.98 at the recombination fraction of 13%.

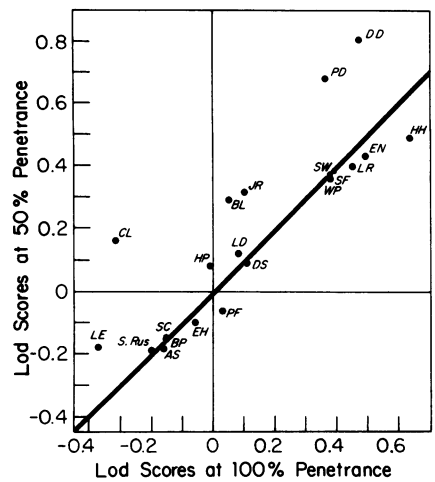


FIGURE 3 Lod scores for each diabetic multiplex family assuming penetrances of 100 and 50% at the most likely recombination fraction of 13%. Only six families (D. D., C. L., J. R., B. L., P. D., and H. P.) contribute a major increase in the lod score at 50% penetrance.

haplotypes and the diabetic phenotype, one of the interpretations of the data considered was that of two different diabetic genes segregating in these families; a dominant gene in those families with one HLA haplotype identical diabetic sibs, and a recessive diabetic gene in those identical for two haplotypes (1). However, in most multiplex families studied the parents were unaffected, a finding compatible with the autosomal recessive mode. The results of our linkage analysis, to our knowledge not reported before in diabetic multiplex families, support the hypothesis of linkage between an autosomal recessive gene, with a role in the pathogenesis of JIDD, and the HLA system. The maximum likelihood estimate, at 50% penetrance, of 13 map units between the JIDD locus and the HLA system reflects odds for linkage of about 9,550:1. The linkage analysis at 100% penetrance resulted in a map distance of 18 units with favorable odds of 832:1.

Our findings should be interpreted in the light of two important considerations: the heterogeneity of JIDD and our strategy of ascertainment. Abundant evidence suggests that JIDD is a genetically heterogenous disorder. Twin studies (4), and segregation analysis done by us (5) and others (6), have strengthened that evidence. This creates problems for genetic analysis. The heterogeneity can be reduced by the methods used in selecting samples, but there is no guarantee that single etiological categories will be produced. The selection of multiplex families will increase the proportion of cases with a significant genetic contribution. The further restriction to multiplex sibships, i.e., excluding those families with affected individuals in more than one generation, will increase the likelihood of autosomal recessive inheritance.

However, these selective strategies should not bias linkage analysis. If the hypothetical diabetes susceptibility gene(s) is not on a chromosome near the marker locus, the marker and test traits still should segregate independently. The sample selection, however, must be taken into account in the interpretation of results. We cannot generalize to all cases of JIDD. Nor can we claim that these selected families represent a single entity. Furthermore, it would be quite premature to use such results for purposes of prediction or genetic counseling, as it has been previously suggested (7).

The choice of a level of penetrance is another difficulty that must be faced when performing linkage analysis. Recently, Rubinstein et al. (7) claimed 50% penetrance, assuming that the susceptibility gene was closely linked to the HLA complex. This conclusion was based on the observation that diabetes had developed in only half of the sibs sharing both haplotypes with the first affected. The presence of affected siblings who did not share both haplotypes was explained by genetic recombination (looser linkage between the loci) or homozygosity on one parent. We do not think it is possible to measure penetrance with such certainty. We have preferred to vary the level of penetrance as a part of the analysis. Thus, the distribution of HLA types and diabetes in our families is compatible either with high penetrance of the diabetes gene(s) and looser linkage to the HLA system, or with lower penetrance and closer linkage. The main effect of lowering the penetrance on the linkage analysis results is to reduce the negative contribution from nonaffected sibs who share both haplotypes with their diabetic siblings. Thus, the different penetrance levels represent different ways of examining the data, and we cannot conclude from these results what the true value of penetrance may be.

A prospective study of these sibships, which is in progress, has revealed abnormalities in carbohydrate metabolism in at least some of the nondiabetic sibs sharing both HLA haplotypes with their diabetic siblings. Thus, the proportion of affected sibs in these families may increase, changing the value of penetrance.

We have analyzed for linkage between JIDD and the HLA using data from Rubinstein et al. (7). We performed this analysis only in the families without HLA recombinants, since the high frequency of recombinants in JIDD families has not been confirmed (1, 8). Furthermore, we excluded another family with a homozygous parent. The autosomal recessive mode and 50% penetrance were assumed. For the 22 families considered, the maximum lod score is 0.88 at a recombination fraction of 13%. The low lod score probably results from a strategy of ascertainment different from ours, with many families including only one diabetic. For the six multiplex families, the maximum lod score is 0.36 at a recombination fraction of 19%. These results are compatible with ours.

## ACKNOWLEDGMENTS

This work was supported by grants 1 RO1-AM20729-01, CA 20531, and General Clinical Research Centers Program (RR-400), The Division of Research Resources, National Institutes of Health.

## REFERENCES

1. Barbosa, J., R. King, H. Noreen, and E. J. Yunis. 1977. The histocompatibility system in juvenile, insulin-dependent diabetic multiplex kindreds. *J. Clin. Invest.* **60:** 989–998.
2. 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.
3. Morton, N. 1955. Sequential tests for the detection of linkage. *Am. J. Hum. Genet.* **1:** 277–318.
4. Nelson, P., D. Pike, A. Cudworth, T. Woodrow, and A. Batchelor. 1975. Histocompatibility antigens in diabetic identical twins. *Lancet.* **II:** 193–194.
5. Barbosa, J., M. Chern, and L. Greenberg. 1978. Segregation analysis of juvenile-onset, insulin-dependent diabetes in 136 families. *Diabetes.* **27:** 458. (Abstr.)
6. Barrai, I., and H. Cann. 1965. Segregation analysis of juvenile diabetes mellitus. *J. Med. Genet.* **2:** 8–11.
7. Rubinstein, P., N. Suciu-Foca, and J. Nicholson. 1977. Genetics of juvenile diabetes mellitus. *N. Engl. J. Med.* **297:** 1036–1040.
8. Barbosa, J., H. Noreen, R. King, and E. Y. Yunis. 1978. Genetics of juvenile diabetes. *N. Engl. J. Med.* **297:** 462.