JCI The Journal of Clinical Investigation

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J Clin Invest. 1980;65(3):592-601. https://doi.org/10.1172/JCI109704.

Research Article

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ABSTRACT We have histocompatibility (HLA) genotyped 28 families with insulin-dependent diabetics in two or more consecutive generations, usually parent and child. This strategy of ascertainment was used to maximize the likelihood of obtaining a homogeneous type of disease within a family, and an autosomal dominant mode of inheritance. 76 diabetics and 169 nondiabetics were studied in these families.

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Received for publication 11 May 1979 and in revised form 2 October 1979.

INTRODUCTION

One of the few seemingly well-established facts about the etiology of insulin-dependent diabetes mellitus (IDDM)[1] is that extensive heterogeneity is present (1–8). Thus, several genetic mechanisms are likely to be involved. These may possibly range from multigenic (with each locus contributing only a small effect) to the major genetic effect of Mendelian modes of inheritance. The recent reports of association between the histocompatibility system (HLA) and IDDM (5, 6) indicate a direct and important effect of the HLA antigens on the risk of the disease. One possible explanation of this association is linkage between the two traits; we have conducted a series of linkage analysis studies to examine these possibilities. Genetic studies of IDDM must be designed to answer a specific research question under circumstances that minimize the "noise" of heterogeneity. We have, therefore, sampled multiplex families (i.e., those families with

[1] *Abbreviations used in this paper*: HLA, histocompatibility system; IDDM, insulin-dependent diabetes mellitus; MG families, families with diabetics in multiple consecutive generations; MS families, families with multiple (two or more) diabetic sibs in one sibship of normal parents; θ, recombination fraction.

J. Clin. Invest. © *The American Society for Clinical Investigation, Inc.* · *0021-9738/80/03/0592/10* $1.00
Volume 65 March 1980 592–601

two or more IDDM members). We first analyzed those IDDM multiplex families with normal parents and two or more affected children, i.e., multiple sib-affected or families with multiple (two or more) diabetic sibs in one sibship of normal parents (MS families). These families were selected to maximize the proportion of cases with an autosomal recessive mode of inheritance. Assuming this genetic mode, when using the lod (the logarithm of odds) score method for linkage analysis, we found the highest total lod score of 3.98 at a recombination fraction of 13%, suggesting the existence of a diabetic locus loosely linked to the HLA in these families; however, these results may have been influenced by the association between the HLA and IDDM (2, 9), as discussed below. Conversely, the same method applied to the families published by Rubinstein et al. (10), reportedly ascertained at random, showed a lod score of 0.88, thus failing to indicate linkage between the HLA and IDDM (9). This suggests that our findings of linkage may be specific for the specially selected families studied by us (1, 9), and may not be applicable to families ascertained at random or according to other criteria. Furthermore, it does not confirm the claim of Rubenstein et al. (10) that all IDDM cases are autosomal recessive.

In this paper we report the results of studies on those multiplex families with cases in two consecutive generations, designated the multiple generational (MG) families. We test the hypothesis that the responsible diabetic gene in such families, selected to maximize an autosomal dominant mode of inheritance, is in linkage with the HLA region. Other genetic mechanisms (autosomal recessive or multigenic) could yield such pedigrees, and the results of our analysis must be examined in light of these possibilities. The possible effects of association on the interpretations of the linkage analysis results are also considered. A total of 28 MG families were studied for HLA antigens A, B, C, D, and glyoxalase, and analyzed for concordance and linkage analysis.

METHODS

Clinical material. A total of 28 families with two or more insulin-dependent diabetics in two or more consecutive generations were studied. Families 201, 224, and 230 have been partially reported before (1, 2). These were A.P., M.R., and S.Ros., respectively, (1). All family members filled out questionnaires that included the following information: age and month of onset of diabetes, history of infections during the 3 mo preceding the onset of the diabetes, ethnic background, family history of diabetes, and personal and family history of other diseases. All diabetics are insulin dependent and the great majority have a history of ketonuria unrelated to acute disease or fasting, which is the best available indicator of insulin dependency. Only three diabetics have onset of disease above age 40 but all three are ketosis prone.

Immunogenetic studies. 44 HLA antigens (A, B, C, and D) were determined in 15 families. These were the last families studied and were ascertained in exactly the same way as the first 13 families typed for only antigens A, B, and C. The serological specificities (A, B, and C) were studied by a standard two-stage dye exclusion microcytotoxicity test (11) by using a panel of 90 well-characterized antisera capable of detecting all defined HLA and most w specificities. HLA D typing for the specificities Dw1, 2, 3, 4, 7, 10, and 11 was performed according to a method described (12). Four lines of homozygous typing cells were used for each of the specificities. Double-normalized relative responses of 35 or less to at least three of the four homozygous typing cells were considered typing responses. Responders generating more borderline responses (35–50 double-normalized relative responses) were repeated to confirm the specificity. HLA Dw5, 6, and 8 were tested by using two homozygous typing cells per specificity. Because these specificities are less well defined and were typed by only two cell lines, the results have not been included in this study. HLA Dw9 homozygous typing cells were not used. The high number of blanks or nontyping responses (31%) in the diabetic families is probably due to the fact that typings for the HLA Dw5, 6, 8, and 9 specificities were not documented. There is no evidence from the literature that these specificities are associated with diabetes. There were no A/B or B/D recombinants. 400 Caucasian controls were used for D typing, and 897 for A and B typing. All controls were blood donors from the Minnesota area.

HLA antigens were assigned without knowledge of the medical history. In families in which the parents of a sibship were unavailable, one or both paternal or maternal haplotypes were deduced from those found in the rest of the family.

Glyoxalase was determined according to published methods (13). Nine recombinants between the glyoxalase and the HLA loci were found (9.5% of the informative meiosis); three of these were diabetic children.

Statistical studies. A preliminary examination of the data for evidence of linkage between HLA and IDDM in the 28 MG families was performed by analyzing the degree of HLA concordance in the affected and normal sibs. As an indication of linkage, one expects to observe a high proportion of concordance in diabetic/diabetic sib pairs, and low proportion of concordance in diabetic/normal pairs. To test linkage under the autosomal dominant mode assumed in this study, only those nuclear families with an affected parent and at least one affected offspring were analyzed by this method. Thus, families 205 and 209 were excluded, and family 230 contributed two of these nuclei. To identify the shared haplotype, the HLA haplotype of the affected parent is compared with those of the affected child. Then the other sibs in the sibship are separated according to whether they display the IDDM trait and the common HLA shared in the affected parent/child pair. For the normal sibs, age at the time of study is also considered and so the normal sibs are grouped into two age classes ($\leq$15 and 16–40 yr). This provides a crude correction for delayed onset of disease. In 12 families with two or more affected sibs per sibship all possible diabetic/diabetic sib pairs were counted for HLA concordance.

The linkage analysis was performed with the aid of the computer program LIPED (14), using the lod score method. This method is the one most often used to study linkage in human pedigrees. It involves the calculation of the ratio of two values of the likelihood of obtaining a given pedigree; the numerator of that ratio is calculated at selected recombination values, and the denominator at a recombination value

of 50%, i.e., no linkage. A positive lod score indicates that the probability of linkage is higher than that of no linkage for the pedigrees under analysis. Data from different pedigrees can be combined by adding the lod scores of all pedigrees at each recombination fraction. The recombination fraction with the maximum total lod score is the most probable value of the distance between the loci. A total lod score >3 is considered statistically significant. The autosomal dominant mode of inheritance was assumed for IDDM in the MG families. The level of penetrance varied from 100 to 10%, but only the results for 50% or higher are reported in detail. The frequency of the dominant gene was estimated by the quantity $(1-\sqrt{1-A/C})$, where A = 0.00189 for the assumed population prevalence (15), and C is the penetrance. In order to compare with the studies of the MS families linkage analysis was also performed on the MG families assuming the autosomal recessive mode. In this case, the frequency of the recessive gene was estimated by $\sqrt{A/C}$. It should be noted, however, that association between the traits under consideration, such as that between the HLA and diabetes, may confound positive results of linkage analysis.

RESULTS

Fig. 1 and Tables I–III show, respectively, the pedigrees of the 28 MG families studied, the individual haplotypes in each family, including those of the subjects married into the families, and the ages of the family members. All families are Caucasian and 96% are of Northern European ethnic background (Scandinavia, Finland, Great Britain, Holland, and Northern Germany). Several families were ascertained for the presence of cases in two generations and do not have a proband. In the following families a proband is identifiable and shown in parentheses: 219(3), 210(4), 204(3), 217(7), 228(3), 222(7), 225(8), 227(3), 231(3), 213(5), 223(12), 205(8), 212(5), 209(4), and 214(5). 26 families have a diabetic parent and at least a diabetic child; 5 have two or three diabetic children. In six families there is also one or more diabetic sibs of the diabetic parent. In two families (205 and 209), instead of parent/child affected, there is an aunt or uncle/niece or nephew pair affected by diabetes. In three families (218, 226, and 230) there are diabetics in three consecutive generations, although only the two youngest were tissue typed in families 226 and 230. In two cases (202[3] and 205[5]), the spouses (who married into the families and were parents of diabetics) had first-degree relatives with IDDM. There are 83 known diabetics in these families (46% females); 76 have been tissue typed (92%). Seven diabetics were unavailable; in only one of these could the HLA type be deduced. 219 nondiabetic family members, blood relatives of the diabetics, were ascertained. 169 (77%) have been tissue typed, 22 (10%) have deduced haplotypes, and 28 (13%) are not deducible. For the 25 families with two generations affected, the age of the diabetics at interview is 46±10 (mean±SD) in the first generation, and 18±8 in the second. The age of the nondiabetics is 50±14 in the first generation, and 20±7 in the second. The age at onset of diabetes is 24±14 (#36) in the first generations, and 9±6 (#35) in the second; this difference in age at onset is statistically significant ($t = 3.8, P < 0.01$). In the three families with three affected generations, the age of the diabetics is 65±13, 44±12, and 21±12, and nondiabetics 62±8, 28±9, and 9±4 in the first, second, and third generations, respectively. The age at onset of diabetes is 29±9, 31±6, and 15±9 for the first, second, and third generations, respectively.

There are 11 haplotypes with A or B antigen blanks (2.5%) and 56 with D blanks (31%). Among the diabetics there are 12 homozygotes for locus A (15.6%), and 9 for locus B (11.7%), and for the nondiabetics, 12.2 and 11.6%, respectively. There are 3% (1/37) Dw3 homozygotes among the diabetics, and 6% (4/63) among the nondiabetics. For Dw4 homozygotes, the percents are 5% (2/37) and 2% (1/63), respectively. These differences are not statistically significant. Table IV shows the frequency of several HLA antigens, including those that have been associated with IDDM in previous studies (1, 5, 6). Antigens A1, B8, B18, and, most clearly, Dw3 and Dw4 show an upward trend among diabetics. The genotypes B8/B15 and, especially, Dw3/Dw4 are also more frequent for the diabetics than for normal controls or the nondiabetic relatives. 92% of the diabetics, 78% of the nondiabetic relatives, and 26% of the normal controls type for Dw3 and/or Dw4. Antigens B7, Bw44 (previously B12), and Dw2 are decreased. Dw2 was found in only 1 of 26 diabetics (family 208, subject 2). Because of biases both from larger families and from ascertainment methods, traditional statistical analysis would not be appropriate and, therefore, was not performed.

In the 26 nuclear MG families examined for HLA concordance, families 202, 204, 224, 229, and 230 have multiple sibs affected. Family 224 has three affected sibs and the other families two. Family 230 has two nuclear families with two affected sibs from three generations. Although the affected father in the first generation has not been typed, the two affected daughters are HLA concordant. The chance of both parents also having the same haplotypes is very small; this would be the only circumstance negating full HLA concordance by descent. Thus, this pair of diabetic sibs was scored as concordant. In the families with two affected sibs, we would expect them to share one of the haplotypes of the affected parent 50% of the time, assuming HLA genes are independently assorted in relation to the presumed IDDM gene. We observed four cases of concordance in the five families. This observation has a 16% chance of occurring under the no-linkage assumption, and thus is not a statistically significant deviation from

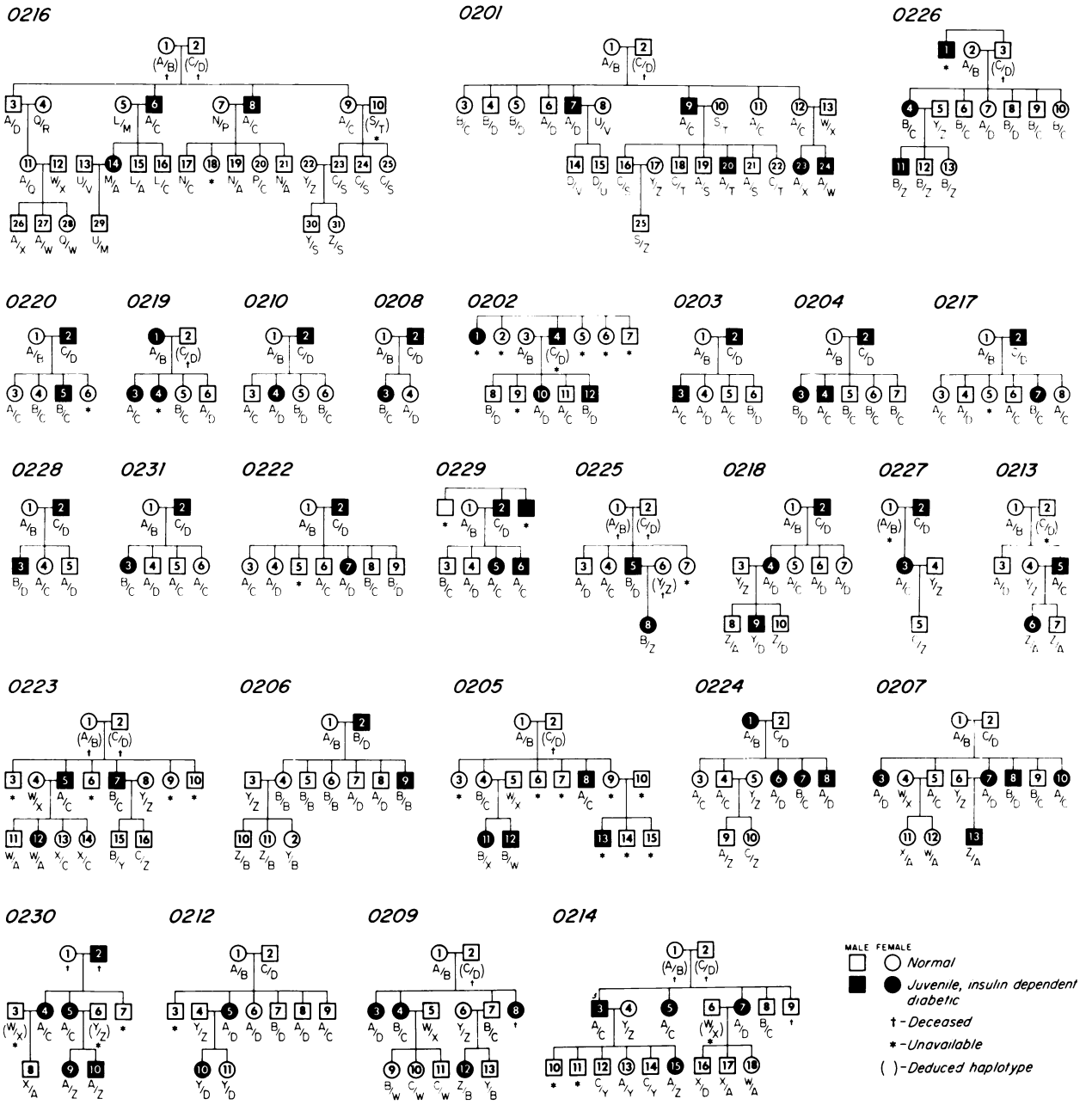

FIGURE 1 Pedigrees of 28 diabetic families with cases in two or more consecutive generations. The maternal HLA haplotypes are designated by the latters A and B, and the paternal by C and D. When more than two generations are tissue typed, the letters U, V, etc., are used to designate additional haplotypes. The number assigned to each subject within a family appears inside the symbols. The ages of the subjects are shown in Table III.

random sampling fluctuation under the null hypothesis. In families with three affected sibs, we expect complete concordance for one parental haplotype 25% of the time; in 75% of the cases one would expect a 2:1 separation. The latter outcome is seen in family 224.

The normal sibs are analyzed by age (≤15 and 16–40 yr) and each one is compared with his/her affected siblings in the same family for concordance of a haplotype from the affected parent. The matching process is repeated as many times as there are affected sibs. The results are shown in Table V. The HLA

TABLE I

*Parental HLA Haplotypes in 28 Diabetic Multiplex Families**

Families	Mother		Father	
	A	B	C	D
216‡	(A2, Bw44)	?	(A1, B40)	(A2, Bw51)
201‡	A1, Bw39	A3, B15	(A1, Bw35)	(A2, X)
226‡	A2, Bw51	A1, B8, Dw3	(A1, B8)	(A2, B8)
220	A1, Bw35, Cw4, F§	A3, B7, F	A1, Bw35, Dw10, F	A29, Bw44, F
219‡	A11, Bw35, Cw4	A26, B15, Cw3	(Aw31, B18)	(A2, B27)
210	A3, B40, Dw4, F	Aw23, Bw44, Dw7, S	A2, B7, F	A3, B8, Dw3, S
208	A3, B7, Cw4, Dw3, S§	Aw23, Bw44, Dw7, S	A1, B8, Dw3, F	A2, B15, Cw4, Dw2, F
202‡	A2, Bw44, F	Aw32, Bw44, Dw1, F	(A3, B15, Cw3, Dw4, S)	(A3, Bw22, Dw4, S)
203	A1, B8, Dw3	Aw23, Bw44, Dw7	A3, B7, Dw4	Aw24, Bw44
204	A1, Bw44, Dw1, F	A2, Bw39, S	A1, B8, Dw3, S	A2, B13, Dw7, S
217	Aw23, Bw44, Dw2, F	A2, B15, Cw3, Dw4, S	Aw24, B27, Cw2, Dw4, S	A2, B7, S
228	A3, B7, F	A1, B7, S	Aw23, Bw35, F	A3, Bw35, Cw4, S
231	Aw24, B15, Cw3, S	A28, B7, Dw5, F	A1, B8, Dw3, F	A1, B17, Dw4, S
222	Aw24, B14, F	A3, B7, Dw1	A2, B7, F	Aw24, B18, F
229	A11, Bw22, Cw1, F	Aw24, B18, S	A2, B15, Cw3, Dw4, F	A2, B27, Cw2, S
225‡	(A3, B7, S)	(Aw24, Bw35, S)	Aw32, B40, Cw2, S	(A2, B40, Cw3)
218	A3, B18, S	X, Bw35, Cw4, Dw1, S	A11, Bw44, Dw4, F	Aw30, B18, S
227‡	(A3, B27)	?	A25, B18, Dw4, S	A2, B8, Dw3
213‡	A25, B18, Dw4, F	A1, B27, Cw2, S	(Aw24, B15, Dw7, S)	(Aw30, B13, Dw11, S)
223‡	(A29, B8, Dw3, S)	(A11, B8, F)	(A3, B8, S)	?
206	Aw24, Bw51, F	A1, B8, Dw3, F	A1, B8, Dw3, F	A29, Bw44, Dw7, S
205‡	A3, Bw35, Cw4	A2, B27, Cw2	(A1, B8)	?
224	A3, B7	Aw24, B18	A2, B40	A3, B7
207	A2, B15, Cw3, Dw4, S	A3, B7, F	Aw24, Bw44, F	A11, Bw35, S
230‖	?	?	?	?
212	Aw32, Bw44, Dw1, S	Aw31, B7, Dw5, S	A2, Bw39, Dw5, F	A1, B8, F
209‡	A1, B8, F	Aw24, Bw35, Cw4, F	(A3, B7, F)	(A2, Bw44, F)
214‡	(Aw32, Bw39, Dw4)	(A28, B40, Dw2)	(A3, B8, Dw3)	(Aw30, B8, Dw3)

* See Fig. 1 for pedigrees.

‡ Deduced haplotypes, parent(s) unavailable or deceased.

§ F, fast glyoxalase; S, slow glyoxalase.

‖ The haplotypes known in this kindred could not be assigned to the deceased members of the first generation (see Fig. 1).

discordance rate is 44% (8/18) among the younger sibs, and 51% (26/51) in the older sibs. Overall, 34 (49%) of the total 69 are HLA discordant. The various rates of discordance in affected and normal pairs are undistinguishable from the random concordance rate of 50%. The nonsignificant higher proportion of HLA concordance in the affected sib pairs, and the lack of a higher proportion of HLA discordance in the normal sibs, is nonconclusive regarding linkage between IDDM and the HLA in the MG families. This method, however, cannot be generalized readily to include the reduced penetrance factor, the multiple generation structure of the pedigree, and the existence of second-order affected relatives, HLA typed or not.

Although a less efficient method to detect linkage in the MG families, the affected sib/sib pair concordance analysis was also performed to compare the results with those obtained by similar analysis in the MS families. Table VI shows this analysis for affected sib/sib pairs in 12 MG families, including 14 different sibships. The HLA haplotypes seem to assort as expected under the no-linkage situation. Also shown are the results of similar concordance analysis done for the MS families that show an HLA haplotype assortment significantly different from that expected (1). The percentages shown for the MS families are different from those reported before (1), because family 224, previously MS, has become MG, i.e., the mother developed IDDM. This was the family that contributed the only two HLA-discordant sib pairs among the MS families. There is a marked difference between the MG and MS families in this analysis. This divergence, however, must be interpreted cautiously because the different ascertainment of these families could have influenced the results.

A more generalized linkage analysis between the HLA and a hypothetical diabetic locus using the LIPED program (14) was applied to the 28 MG

TABLE II
*HLA Haplotypes of Individuals Married into the 28 Diabetic Multiplex Families**

Families	Subjects	Haplotypes
216	4	A2, B40 / X, B7, Cw2
	5	A2, B17, Dw4 / Aw32, Bw51, Cw2, Dw11
	7	Aw24, Bw38 / A1, Bw22, Cw3
	10	(Aw24, B18 / ?)
	12	A2, B18 / A3, Bw49
	13	A2, Bw39, Dw1, F / X, B12, Dw4, S
	22	Aw32, B18, Cw4 / A3, B15
201	8	Aw32, B15 / A25, Bw44, Dw7
	10	A2, B8, Dw3 / A2, B15, Dw4
	13	A2, B8 / Aw24, B7
	17	(A11, B15, Cw3 / ?)
226	5	A1, B27, Dw1 / A2, B15, Cw3, Dw4
225	6	(A28, Bw44 / ?)
218	3	A2, B15, Dw4 / A26, B15, Dw5±
227	4	Aw24, Bw44, Dw2, S / A10, X, F
213	4	A3, B7, Dw2, F / A3, B7, F
223	4	A1, B8, F / A11, B18, Dw5±, F
	8	A25, B18, F / A2, B7, S
206	3	Aw24, Bw35, Cw4 / A1, B17
205	5	A2, B15, Cw3, Dw4, F / Aw24, B40, Cw3, S
224	5	Aw24, B18 / A2, Bw51
207	4	A1, B8, S / Aw24, Bw35, Cw3, S
	6	A3, B18, S / A28, B27, Cw2, S
230	3	(A3, B18 / ?)
	6	(? / Aw24, B40)
212	4	A3, Bw35, Cw4, Dw1 / A1, B18, Dw5
209	5	A2, B7 / Aw30, B18, Cw2
	6	A2, Bw44, Dw4 / A29, Bw44, Dw7
214	4	A2, Bw44, Dw4, S / A3, B7, F
	6	(x, B14, Dw7, S / A2, B40, Cw3, Dw2, F)

* See Fig. 1 for pedigrees and Table I for explanations.

pedigrees, assuming the autosomal dominant mode. The families with only one diabetic parent and one diabetic child, however, do not contribute much to this analysis because the diabetic must share one haplotype. The results, as shown in Fig. 2, indicate low total lod scores across all values of recombination fractions at 100, 90, 70, and 50% penetrance. The gene frequencies are 0.00095, 0.00105, 0.00135, and 0.00189, respectively. The peak of the total lod score curve increased and moved toward a smaller recombination fraction as the level of penetrance decreased. For the 50% penetrance, the maximum total lod score is 0.37 at a recombination fraction (θ) of 0.29. Fig. 3 shows the lod scores at 50% penetrance for each family. This penetrance is suggested by the 50% concordance rate for IDDM reported in identical twins (3), although we recognize that it may not be pertinent to our selected sample. Only family 201 has a clearly positive result with the highest lod score of 1.53 at $\theta = 0$.

Because the actual penetrance of the diabetic gene(s) is not known we also studied other penetrance levels. Linkage analysis results at 40, 30, 20, and 10% penetrance show a steady increase of the maximum lod scores (0.47, 0.57, 0.68, and 0.78) at smaller θ values (0.26, 0.23, 0.20, and 0.18), but none of the total lods approaches statistical significance. Linkage analysis of the MG families, assuming the autosomal recessive mode of inheritance, yielded a maximum lod score of 0.33 at $\theta = 0.27$ and 50% pentrance, which is much lower than what one would expect had the 28 MG families been selected for the same genetic mode as the MS families. Because the MG families with more than one affected sib (MS type of family), and families with three consecutively affected generations are more likely to be informative for linkage analysis, the total lod score for such families (202, 204, 218, 224, 229, and 230) was separately calculated. The maximum score was 0.26 at $\theta = 0.25$ and penetrance of 50%, not different from the score obtained for all 28 MG families.

The data on glyoxalase did not provide sufficient informative matings for linkage analysis.

DISCUSSION

The results of concordance and linkage analysis between the HLA and the IDDM trait in our 28 MG families, although nonconclusive, are interesting when contrasted with those we have obtained for the MS families (1, 9). The latter were ascertained to maximize the likelihood of dealing with a genetically homogeneous form (or forms) of autosomal recessive IDDM. In such a subset of patients (about 11% of all families with one child affected.),[2] we presented evidence for the existence of linkage of ~13 map units between the HLA and the assumed diabetic locus (9). On the other hand, the MG families (about 5% of all families with one child affected)[2] ascertained to maximize the likelihood of a genetically homogeneous form (or forms) of autosomal dominant IDDM, fail to show linkage with the HLA. Several possibilities may be relevant to these results.

First, the diabetes in the MG families may not depend on an autosomal dominant gene as we had postulated. It is possible that our MG sample includes forms of diabetes that are caused by the combined small effects of a number of genetic and environmental factors, i.e., multifactorial. Linkage analysis between a marker and a multifactorial trait is expected to give nonconclusive results.

Second, the diabetes in the MG families may indeed depend on a major autosomal gene. The mode of

[2] Chern, M. M., E. Anderson, and J. Barbosa. Manuscript in preparation.

TABLE III

Ages of the Family Members Shown in Fig. 1

Families	Subject number (age)*
216	1(96), 2(88), 3(61) 4(61), 5(59), 6(57), 7(55), 8(55), 9(53), 10(53), 11(35), 12(35), 13(31), 14(30), 15(26), 16(24), 17(25), 18(22), 19(17), 20(8), 21(8), 22(32), 23(32), 24(26), 25(24), 26(14), 27(9), 28(5), 29(3), 30(14), and 31(10)
201	1(82), 2(72), 3(64), 4(63), 5(61), 6(57), 7(57), 8(56), 9(55), 10(55), 11(52), 12(44), 13(44), 14(17), 15(13), 16(29), 17(30), 18(26), 19(22), 20(19), 21(16), 22(14), 23(12), 24(11), and 25(3)
226	1(60), 2(60), 3(46), 4(39), 5(40), 6(34), 7(28), 8(27), 9(22), 10(22), 11(15), 12(13), and 13(8)
220	1(36), 2(36), 3(13), 4(12), 5(8), and 6(2)
219	1(55), 2(56), 3(27), 4(26), 5(25), and 6(25)
210	1(35), 2(34), 3(12), 4(11), 5(3), and 6(2)
208	1(48), 2(47), 3(18), and 4(15)
202	1(55), 2(52), 3(49), 4(49), 5(46), 6(44), 7(39), 8(27), 9(24), 10(20), 11(17), and 12(13)
203	1(44), 2(46), 3(17), 4(15), 5(13), and 6(10)
204	1(46), 2(45), 3(21), 4(20), 5(19), 6(18), and 7(17)
217	1(56), 2(56), 3(29), 4(28), 5(26), 6(25), 7(23), and 8(20)
228	1(44), 2(47), 3(22), 4(20), and 5(14)
231	1(52), 2(52), 3(30), 4(28), 5(21), and 6(16)
222	1(56), 2(56), 3(32), 4(29), 5(27), 6(25), 7(23), 8(20), and 9(14)
229	1(40), 2(40), 3(20), 4(18), 5(14), and 6(11)
225	1(92), 2(89), 3(61), 4(59), 5(57), 6(54), 7(54), and 8(25)
218	1(51), 2(55), 3(32), 4(30), 5(29), 6(25), 7(19), 8(10), 9(8), and 10(3)
227	1(57), 2(56), 3(29), 4(30), and 5(3)
213	1(56), 2(64), 3(35), 4(32), 5(32), 6(8), and 7(4)
223	1(66), 2(66), 3(53), 4(52), 5(51), 6(49), 7(47), 8(48), 9(46), 10(42), 11(25), 12(23), 13(21), 14(17), 15(19), and 16(18)
206	1(52), 2(55), 3(30), 4(29), 5(27), 6(26), 7(23), 8(19), 9(11), 10(6), 11(5), and 12(3)
205	1(84), 2(84), 3(61), 4(60), 5(68), 6(57), 7(55), 8(52), 9(42), 10(42), 11(28), 12(24), 13(19), 14(17), and 15(14)
224	1(61), 2(60), 3(30), 4(28), 5(30), 6(24), 7(22), 8(21), 9(5), and 10(4)
207	1(57), 2(58), 3(34), 4(36), 5(31), 6(29), 7(28), 8(25), 9(22), 10(19), 11(11), 12(8), and 13(7)
230	1(77), 2(33), 3(55), 4(55), 5(53), 6(59), 7(50), 8(15), 9(33), and 10(28)
212	1(62), 2(62), 3(32), 4(30), 5(30), 6(28), 7(26), 8(12), 9(11), 10(9), and 11(5)
209	1(74), 2(59), 3(50), 4(48), 5(48), 6(44), 7(44), 8(33), 9(22), 10(20), 11(18), 12(16), and 13(15)
214	1(80), 2(89), 3(50), 4(51), 5(49), 6(48), 7(47), 8(45), 9(40), 10(26), 11(25), 12(23), 13(22), 14(19), 15(9), 16(22), 17(18), and 18(17)

* Ages in years at time of study or time of death.

inheritance of this gene is more likely to be dominant, because in 26 of the 28 families we observed parent to child transmission of the trait. When the likelihood of each pedigree in the sample is evaluated under the Mendelian modes of inheritance in a crude manner, i.e., without correcting for bias of ascertainment, the odds favor the autosomal dominant over the autosomal recessive mode of inheritance (~10:1) in almost all MG families. The nonconclusive linkage results would then imply that the dominant IDDM gene is either on chromosome 6, but too distant from the HLA to show evidence for linkage, or is on another chromosome. Family 201, with a lod score of 1.53 at $\theta = 0$, certainly suggests the possibility of a dominant gene linked to the HLA.

Third, it is possible that the diabetic MG families are genetically less homogeneous than the MS families, because several generations are involved, and individuals marrying into the families may bring in additional genetic liability for diabetes. In some of the families studied these spouses had a family history of IDDM. However, a test of heterogeneity on the linkage data, using the method reported by Morton (16), failed to show significant heterogeneity ($\chi^2 = 18.55$, degrees of freedom = 27, $P < 0.75$).

Fourth, it may be that the MS and MG families are not genetically different from each other. However, the linkage analysis, assuming the autosomal recessive mode, applied to the MG families yielded a much weaker evidence for linkage than in the MS families (MG: total lod = 0.26, $\theta = 0.25$; MS: total lod = 3.98, $\theta = 13$). This seems to indicate genetic dissimilarity between the two samples although it is possible that both groups of families are heterogeneous. Further detection of genetic differences between these two samples may have to come from other sources of information. Indeed, there may be differences between the MS and MG families for HLA

TABLE IV

HLA Antigen Frequencies in Diabetic and Nondiabetic Members of the Diabetic Multiplex Families

Antigen	Controls (897)*	Diabetics (76)	Nondiabetics (169)
	%	%	%
Locus A			
1	27	40	37
2	55	44	46
3	28	35	34
11	10	12	5
w24	15	22	27
w30	4	5	4
Locus B			
7	26	19	24
8	21	39	32
w44‡	30	21	26
15	17	23	16
18	6	17	18
27	5	12	6
40	18	12	13
w35	14	19	20
w39	7	13	10
8/15	1	6	2
8/40	3	3	1
Locus D	(400)*	(37)*	(63)*
w2	18	4	17
w3	15	61	49
w3/w4	2	34	8
w4	12	66	37

* Number of controls or family members.

‡ Known previously as B12.

antigen frequencies. In the MS families, B8 was present in 31%, and B15 in 36% when all diabetics were considered. Analysis for only the oldest diabetic sib in each family yields 33% for B8, 42% for B15, and 9% for B8/B15. These frequencies seem to be different from those shown for the MG families (Table IV). When only analyzing for the diabetic parent in each MG family, the frequencies are 46% for B8, 21% for B15, and 4% for B8/B15. These differences, however, must be interpreted cautiously because ascertainment biases may have influenced the results.

TABLE V

Analysis of HLA Haplotype Concordance for Affected/Unaffected Sib Pairs in Relation to the Haplotype Shared by the Affected Parent/Affected Child Pair

	Age at onset (yr)		
	≤15	16–40	Total
Sharing one HLA haplotype	10	25	35
Not sharing one HLA haplotype	8	26	34
Total	18	51	69

TABLE VI

HLA Haplotype Concordance for Diabetic Pairs in Sibships with Two or More Diabetics in MG and MS Families*

Number of concordant haplotypes	Pairs of diabetic sibs: Concordance found: MG families (23)‡	Pairs of diabetic sibs: Concordance found: MS families (35)‡	Concordance expected
	%		%
2	30 (7)‡	57 (20)	25
1	48 (11)	43 (15)	50
0	22 (5)	0	25

* The MS families have been reported (1). Changes in sib pair concordance reflect the fact that family 224, previously an MS family, has become an MG family (mother developed IDDM).

‡ Number of pairs in parentheses.

In spite of the nonconclusive results for linkage and concordance analysis, the frequencies of the HLA antigens B8, Dw3, and Dw4 are increased among the diabetics in the MG families, indicating the existence of an important HLA-associated genetic factor for diabetes in these families. The risk seems to be particularly high for the genotype Dw3/Dw4. Associations of IDDM with the genotypes B8/B15 and Dw3/Dw4, stronger than what would be predictable from the associations with those antigens separately, have been

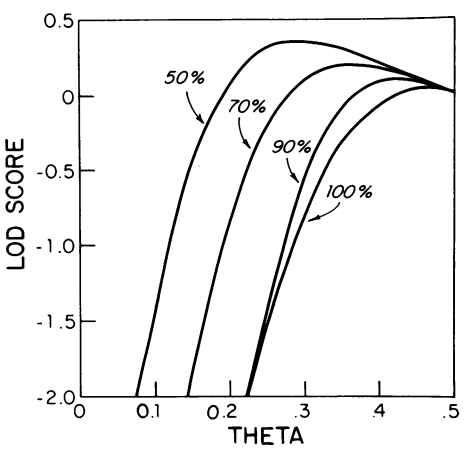

FIGURE 2 Total lod scores for linkage between diabetes and the HLA in 28 families with cases in two or more consecutive generations, assuming the dominant mode, penetrances of 100, 90, 70, and 50%, and the respective gene frequencies of 0.00095, 0.00105, 0.00135, and 0.00189. The total lod score (0.37) is highest for 50% penetrance, although not significant. Theta, recombination fraction.

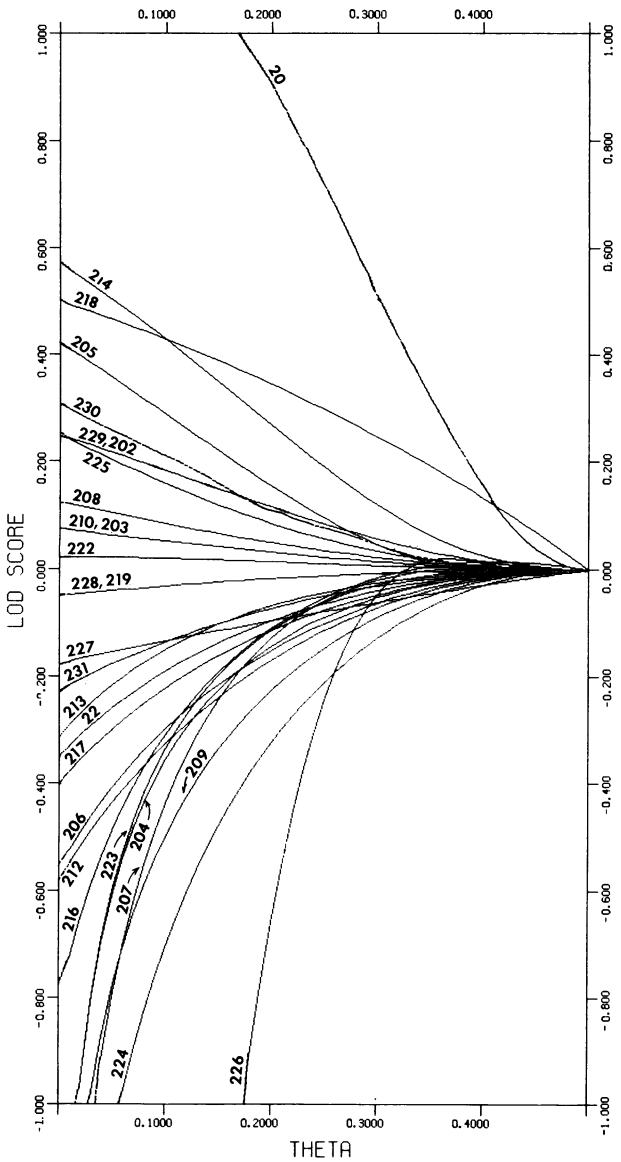

FIGURE 3 Family lod scores for linkage between diabetes and the HLA in 28 families with cases in two or more consecutive generations, assuming the dominant mode, 50% penetrance, and gene frequency of 0.00189. The highest total lod score is 0.37 (NS) at the recombination fraction of 0.29. Theta, recombination fraction.

described before (5, 6). Recently, Christy et al. (8) have reported studies of multiplex and simplex diabetic Danish families typed for HLA A, B, and D. They also find a high risk for the disease associated with the genotype Dw3/Dw4, which is especially striking in the multiplex families. In their data Dw4 is mostly increased in diabetics from the latter families. It is of interest that in our small population study of D antigens in IDDM, there were only 20% of diabetics from multiplex families, and the frequencies of Dw4 and Dw3/Dw4 were normal (7), which is in striking contrast to the excess seen in both the Danish families and those reported here. Furthermore, Christy et al. (8) suggest that Dw4 is primarily a characteristic of IDDM with onset before age 15. Indeed, in a large population study of HLA A and B antigens, which we have performed,[3] B15 (which is known to be in linkage disequilibrium with Dw4) is increased in frequency only in patients with onset of disease below age 10. In the families reported here, however, Dw4 was not more frequent in the diabetics with earlier age of onset. Thus, both ours and the Danish data seem to offer new evidence for extensive heterogeneity of IDDM. The high risk for the Dw3/Dw4 heterozygotes has been interpreted as a demonstration of overdominance. Our results for the MG families are compatible with such an explanation. Whether this high risk for IDDM in subjects carrying the genotype Dw3/Dw4 depends on IDDM alleles in strong linkage disequilibrium with Dw3 and Dw4, or is due to the D alleles themselves, is not known.

Our ascertainment scheme may also have resulted in selective sampling of highly susceptible individuals (with Dw3/Dw4 genotypes), instead of a sample of families with autosomal dominant inheritance as we had assumed. Another methodological problem pertains to linkage analysis in the presence of association. If the association is due to linkage disequilibrium as a result of tight linkage and selection, then a high lod score for linkage should be obtained for informative families, regardless of the method of ascertainment and the presence of ascertainment biases. Conversely, if the association reflects a pleiotropic effect of the involved HLA antigens, the lod score for linkage could be artifically inflated. Thus, results for positive linkage between the HLA and IDDM should be reported with caution. Our earlier results on loose linkage between the HLA and IDDM in the MS families are indeed subject to this qualification. Such a situation, however, does not apply to the MG families, for whom the linkage is nonconclusive.

The near absence of Dw2 among IDDM patients has been reported before (5). We have typed to date 78 diabetics for D alleles, 37 from this study and 41 from a population study (7). We have found only one diabetic positive for Dw2. Whether this strikingly decreased risk for Dw2 subjects reflects a genetic protective factor for IDDM or other mechanism is not known.

The age at onset of disease in the youngest generation is significantly lower in the MG families. This phenomenon (known as anticipation) usually results from the incomplete life experience in younger

[3] Barbosa, J., R. Ramsay, and M. Chern. Submitted for publication.

generations. As the currently unaffected are followed through time, some will develop diabetes at older ages, and these cases (when averaged with the others) will increase the age at onset. Whatever the genetic mechanism reflected in the high HLA antigen frequencies, it is unlikely to explain the anticipation effect because Dw3/Dw4 diabetic subjects did not differ significantly for age at onset of disease from their diabetic relatives with other genotypes.

In conclusion, the discrepancy in the linkage and concordance analysis and in HLA antigen frequencies between the MS and MG families is compatible with a genetic difference between these two types of families and seems to confirm the theory of genetic heterogeneity in IDDM. As stated above, however, the differences between MS and MG families could be due to ascertainment biases. The high frequency of Dw3, Dw4, and the genotype Dw3/Dw4 in the MG families confirms the importance of genetic factors in the D region of the HLA for the pathogenesis of IDDM, and is compatible with the theory of overdominance (12, 13). Further progress in our ability to sort out the different pathogenetic types of IDDM, including the confirmation of differences between MS and MG families, may depend on the availability of additional biochemical and immunological indicators of the diabetic syndrome.

ACKNOWLEDGMENTS

This work was supported by U.S. Public Health Service grant 1 RO1-AM-20729, contract NO1-HD-82846, and General Clinical Research Centers Program (RR-400), 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. Barbosa, J., and M. M. Chern. 1979. On the genetic heterogeneity of juvenile hyperglycemia. *In* Treatment of Early Diabetes. R. A. Camerini-Davalos and B. Hanover, editors, Plenum Publishing Corp., New York. 51–57.
3. Nelson, P., D. Pike, A. Cudworth, T. Woodrow, and A. Batchelor. 1975. Histocompatibility antigens in diabetic identical twins. *Lancet.* **II:** 193–194.
4. Rotter, J., and D. Rimoin. 1978. Heterogeneity in diabetes mellitus. Update 1978. *Diabetes.* **27:** 599–605.
5. Nerup, J. 1978. HLA studies in diabetes mellitus: a review. *Adv. Metab. Disord.* **9:** 263–277.
6. Cudworth, A., and H. Festenstein. 1978. HLA genetic heterogeneity in diabetes mellitus. *Br. Med. Bull.* **34:** 285–289.
7. Barbosa, J., M. Chern, N. Reinsmoen, H. Noreen, R. Ramsay, and L. Greenberg. 1979. HLA-Dw antigens in unrelated juvenile, insulin-dependent diabetes. *Tissue Antigens.* **14:** 426–436.
8. Christy, M., A. Green, B. Christau, H. Kromann, J. Nerup, P. Platz, M. Thomsen, L. Ryder, and A. Svejgaard. 1979. Studies of the HLA system and insulin dependent diabetes mellitus. *Diabetes Care* **2:** 209–214.
9. Barbosa, J., M. M. Chern, H. Noreen, V. E. Anderson, and E. J. Yunis. 1978. Analysis of linkage between the major histocompatibility system and juvenile, insulin-dependent diabetes in multiplex families. *J. Clin. Invest.* **62:** 492–495.
10. Rubinstein, P., N. Suciu-Foca, and J. Nicholson. 1977. Genetics of juvenile diabetes mellitus. *N. Engl. J. Med.* **297:** 1036–1040.
11. Amos, D., W. Bashir, W. Boyle, M. MacQueen, and A. Tiilikainen. 1969. A simple microcytotoxicity test. Transplantation *(Baltimore).* **7:** 220–223.
12. Reinsmoen, N., H. Noreen, P. Friend, E. Giblett, L. Greenberg, and J. Kersey. 1979. Anomalous mixed lymphocyte culture reactivity between HLA A, B, C, DR identical siblings. *Tissue Antigens.* **13:** 19–34.
13. Kompf, J., S. Bissbort, S. Gussman, and H. Ritter. 1975. Polymorphism of cell glyoxalase I (E.C.: 4.4.15). A new genetic marker in man. *Humangenetik.* **27:** 141–143.
14. 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.
15. Kyllo, C., and Nuttall, F. 1978. Prevalence of diabetes mellitus in school age children in Minnesota. *Diabetes.* **27:** 57–60.
16. Morton, N. 1955. Sequential tests for the detection of linkage. *Am. J. Hum. Genet.* **1:** 277–318.