Is Juvenile Diabetes Determined by a Single Gene Closely Linked to HLA?

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SUMMARY

The transmission behavior of insulin-dependent juvenile diabetes mellitus (JDM) has been studied with respect to its frequency in the relatives of JDM probands and its possible linkage to the HLA complex. Mathematical analysis shows that under a single locus hypothesis a very restricted range of incidence rates is possible in the full siblings of probands once the concordance rate in monozygotic (MZ) twins is specified. Specifically, for a given population prevalence of the disease, high concordance rates in MZ twins require high incidence rates in siblings, and low rates require low incidence rates, if a single locus model is to be valid. Moreover, if these rates do conform to a single locus model, then they give additional information about possible linkage between the purported JDM susceptibility gene and the HLA complex. By using observations on the identity by descent scores at the HLA locus of sibling pairs, both of whom are affected with JDM, it is shown that tight linkage of a disease susceptibility locus is possible only when the MZ twin and sibling incidence rates are low, whereas high rates support loose linkage. If the single locus model is rejected, then an alternative hypothesis, involving epistasis between a JDM susceptibility locus and genes in (or close to) the HLA complex can be suggested as a mechanism whereby JDM would appear to be linked to HLA within families while maintaining an association with HLA at the population level. DIABETES 28:527–532, June 1979.

Few diseases are as heterogeneous as diabetes. Indeed, the heterogeneity is so pervasive that attempts to characterize it genetically are, in the words of J. V. Neel, "a geneticist's nightmare."[1,2] With respect to type I insulin-dependent juvenile diabetes mellitus (JDM), two single locus hypotheses have recently been advanced. Rubinstein et al.,[3] Thomson and Bodmer,[4] Barbosa et al.,[5] and Suarez and Hodge[6] advanced an incompletely penetrant recessive hypothesis, while Spielman et al.[7,8] and Svejgaard et al.[9] advanced an incompletely penetrant dominant hypothesis. In some of the above studies, the alleged JDM locus is thought to be closely linked to the HLA complex—especially the D locus—and in linkage disequilibrium with certain haplotypes, thereby giving rise to the HLA associations observed in most populations.[10–14] In others,[5,6] the linkage is thought to be loose.

In contrast with these two single locus hypotheses is the suggestion of Rotter and Rimoin[15] that intra-JDM heterogeneity is so extensive that a single genetic mechanism is unlikely. In particular, Rotter and Rimoin postulate that there are at least two distinct forms of JDM—one type associated with the HLA antigen B8 and the other type associated with BW15. Their survey of the literature suggests that the former type of JDM is characterized by an increased association with DW3, absence of anti-insulin antibodies, increased islet cell antibodies, and increased antipancreatic cell-mediated immunity, whereas the latter type of JDM is characterized by an increased association with CW3, high production of insulin antibodies, and no increase in islet cell antibodies or antipancreatic cell-mediated immunity.

The purpose of this paper is to report the results of a single locus analysis of JDM. In so doing, we treat the disease *as if it were* determined by a major single locus. This assumption, while temporarily ignoring the possible heterogeneity, will allow the transmission behavior of the disease to be explored with respect to both its frequency in the relatives of probands and its possible linkage to HLA. It will be shown that under the constraints of the single locus model, the incidence of JDM in one class of relatives, monozygotic (MZ) twins, predicts a very narrow incidence range in other classes of relatives, specifically in full siblings. Additionally, since observed HLA–JDM associations have been interpreted as resulting from linkage disequilibrium (thereby

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implying tight linkage), it will be shown that only a limited range of incidence rates is compatible with the hypothesis of tight linkage, whereas other sets of incidence rates are compatible with loose linkage. Consequently, these incidence rates in relatives can be used to approach two crucial questions concerning the genetics of JDM: (a) If they are not consistent with the single locus model, then that model can be rejected, (b) if they are, then they can help to rule out either tight or loose linkage within the single-locus model. Since the incidence rates in MZ twins and in siblings have both been reported over wide ranges of values, these questions cannot be answered until more reliable data are available.

MODELS AND METHODS

Incidence rates. We restrict our treatment of single locus models to those with two alleles, A and a, with respective population frequencies p and q = 1 − p. The genotypes AA, Aa, and aa occur with frequencies p^2, 2pq, and q^2, and their probabilities of manifesting JDM are f_1, f_2, and f_3, respectively. Thus the model is constrained by the population prevalence (K) of the disease such that

$$K = p^2f_1 + 2pqf_2 + q^2f_3.$$

The f_i's (referred to as the penetrance vector) are allowed to assume any values in the range zero to unity. Under this model, the probability that a person is affected is a function of only that individual's genotype and is not correlated with that of other family members, above and beyond the correlation present for genotypes.

Since we are interested in the incidence of JDM in the relatives of JDM probands, it is convenient to parameterize the model by the locus' additive variance (V_A) and its dominance variance (V_D)[16]:

$$V_A = 2pq[q(f_3 - f_2) + p(f_2 - f_1)]^2$$

$$V_D = p^2q^2(f_1 - 2f_2 + f_3)^2.$$

The incidence of JDM in different classes of relatives (R_i) can now be expressed as[17]

$$R_i = K + (x_iV_A + y_iV_D)/K \quad (1)$$

where x_iV_A and y_iV_D are the appropriate weighted proportions of the additive variance and the dominance variance, respectively, for that class of relatives.

Since juvenile insulin-dependent diabetes, before the discovery and widespread use of insulin, was essentially a lethal phenotype, observations of its incidence in the parents of JDM probands are most likely biased downward (especially in older studies). Consequently, we will restrict our analysis of incidence rates to the MZ co-twins (R_M) and full siblings (R_S) of JDM probands. In terms of the model's parameters, the incidence rates in Eq. 1 become

$$R_M = K + (V_A + V_D)/K \quad (2)$$

and

$$R_S = K + (\tfrac{1}{2}V_A + \tfrac{1}{4}V_D)/K. \quad (3)$$

From Eqs. 2 and 3 it is seen that the incidence rates are linear in V_A and V_D. Thus for MZ co-twins and full siblings of probands, any particular incidence rate can be described as a straight line in the V_A, V_D plane. For MZ twins and full siblings the respective regressions are

$$V_D = K(R_M - K) - V_A \quad (4)$$

and

$$V_D = 4K(R_S - K) - 2V_A. \quad (5)$$

Because of the constraints imposed by the population prevalence of JDM, there are derivative constraints on V_A and V_D. For any value of K, the compatible values of V_A and V_D can be determined with the methods of Suarez et al.[18] Figure 1 shows the envelope of such compatible values for the case K = 0.0016.

To convert the V_A − V_D constraints to constraints on the incidence rates R_M and R_S, superimpose the appropriate regressions according to Eqs. 4 and 5 onto the envelope in Figure 1; the results are shown in the enlargement of Figure 1 for all values of R_M less than 60%. Figure 2 then plots R_M vs. R_S directly and shows the narrow range of R_S values permissible for each given R_M value.

Linkage to HLA. When the mode of transmission is known, the optimal approach to linkage analysis is maximum likelihood. However, when the mode of transmission is unknown, as is the case for JDM, it is more convenient to use the af-

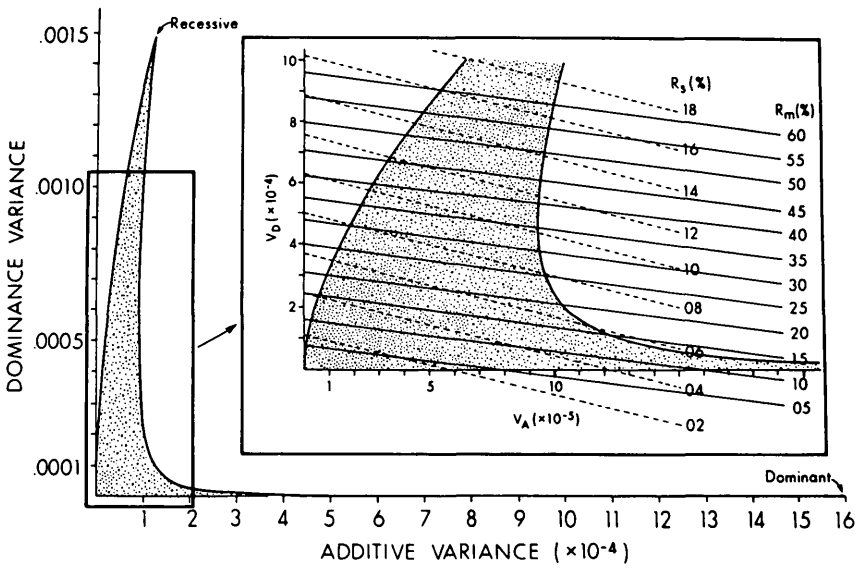

FIGURE 1. The acceptable region of the single locus model for a population prevalence of JDM of 0.16%. The envelope (stippled region) encloses all combinations of the dominance variance (V_D) and additive variance (V_A) consistent with the model. Enlargement: Values of V_D and V_A that give rise to the specified incidence of the disease in full siblings (R_S) of JDM probands (dashed lines) and monozygotic (MZ) twins (R_M, solid lines). Joint incidence rates lying significantly outside the envelope imply that the single locus hypothesis is unacceptable.

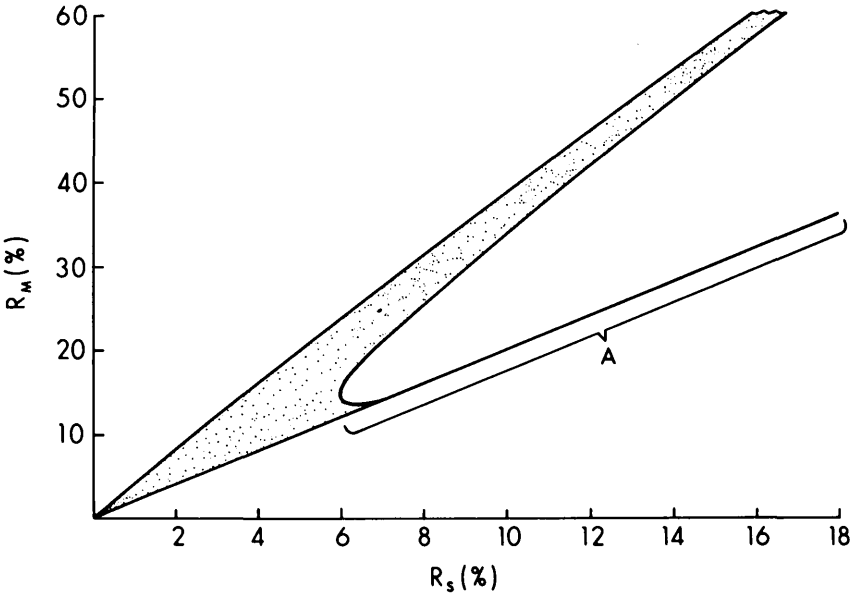

FIGURE 2. Joint concordance rates in monozygotic co-twins (R_M) and full siblings (R_S) of JDM probands that are consistent with the generalized single locus model. The line labeled A corresponds to the narrow region lying along (and immediately above) the abscissa of Figure 1 with $V_A > 2 \times 10^{-4}$.

fected sibling pair approach.[19,20] The advantage of using this approach is that an investigator needs good estimates of only K, V_A, and V_D, rather than of the four parameters (q, f_1, f_2, and f_3) that define the segregation distributions and are, in general, more difficult to estimate accurately.[21] The affected sibling pair method uses perturbations in the distribution of identity by descent (IBD) scores at a marker locus to detect the presence of a linked disease susceptibility locus. In the absence of linkage, the probability that two siblings share neither, one, or both alleles IBD at the marker locus is independent of the siblings' disease phenotypes. This probability distribution is simply ¼, ½, and ¼ for IBD = 0, 1, and 2, respectively. However, when the disease susceptibility locus is linked to the marker locus, the IBD distribution at the latter locus is no longer independent of the siblings' phenotypes. The largest perturbation in the IBD distribution is found to occur in sibling pairs in which both members are affected with the disease. The magnitude of the perturbation depends on both the closeness of the two loci and the magnitude of the genetic effect, *i.e.*, $(V_A + V_D)/[K(1 - K)]$, which ranges from zero when $f_1 = f_2 = f_3$ to unity when the disease is Mendelian.[22] The affected sibling pair IBD distribution is given by the following three equations, derived in Suarez et al.:[20]

$$\Pr(\text{IBD} = 2) = \frac{1}{4} + \frac{(\Psi - \tfrac{1}{2})V_A + (\Psi^2 - \tfrac{1}{4})V_D}{4(K^2 + \tfrac{1}{2}V_A + \tfrac{1}{4}V_D)}, \quad (6)$$

$$\Pr(\text{IBD} = 1) = \frac{1}{2} - \frac{2(\Psi^2 - \Psi + \tfrac{1}{4})V_D}{4(K^2 + \tfrac{1}{2}V_A + \tfrac{1}{4}V_D)}, \quad (7)$$

$$\Pr(\text{IBD} = 0) = \frac{1}{4} - \frac{(\Psi - \tfrac{1}{2})V_A + (2\Psi - \Psi^2 - \tfrac{3}{4})V_D}{4(K^2 + \tfrac{1}{2}V_A + \tfrac{1}{4}V_D)}. \quad (8)$$

Ψ is a linkage parameter, defined as $\Psi = \theta^2 + (1 - \theta)^2$, where θ denotes the recombination fraction between the disease susceptibility and marker loci. When $\Psi = 1$, θ is zero and the two loci are completely linked, i.e., recombination never occurs between them. When $\Psi = \frac{1}{2}$, $\theta = \frac{1}{2}$ and the two loci are unlinked, i.e., recombination occurs 50% of the time.

RESULTS

To investigate the familial distribution of JDM *if* it is determined by a major single locus, we will assume the disease has a population prevalence of K = 0.16%.[23] An accurate estimate of JDM's prevalence is difficult to make owing to its relatively low frequency (hence requiring a very large sample size), its probable bimodally distributed age of onset in persons under 30 yr of age[24] (requiring demographic trends in a population's age structure to be taken into account), and the very real possibility that at least some middle-age onset cases are etiologically identical to juvenile-onset cases. It is unlikely, however, that the estimate of K = 0.16% differs from the true prevalence by more than 50%, since other reported estimates closely bracket this figure.[25-27] The major conclusions of the analysis reported here, however, are unaltered even if the true population prevalence is as high as 0.5%. This was established by varying the assumed population prevalence.

Incidence rates. Figure 1 shows the envelope enclosing all values of the additive and dominance variances that are consistent with the model for a population prevalence of 0.16%. The two Mendelian points are also shown—recessive: ($f_1 = f_2 = 0$, $f_3 = 1$, and $q = \sqrt{K}$), which corresponds to ($V_D = 1.476 \times 10^{-4}$, $V_A = 1.229 \times 10^{-4}$), and dominant: ($f_1 = f_2 = 1$, $f_3 = 0$, and $q = 1 - \sqrt{1 - K}$), which corresponds to ($V_D = 6.394 \times 10^{-6}$, $V_A = 1.598 \times 10^{-3}$). Note that the envelope narrowly extends along the abscissa to a point where the trait behaves as a Mendelian dominant. Throughout this region the dominance variance is zero or negligibly small. Since it is unlikely that the concordance rate in MZ twins is greater than 60%, we have chosen to explore the behavior of the model for all possible values of $R_M \leq 60\%$ (Figure 1, enlargement, and Figure 2). From Eq. 4 we plotted concordance rates for MZ twins (in increments of 5%) and from Eq. 5, incidence rates for full siblings (in increments of 2%). For any particular concordance rate in MZ twins, only a restricted incidence range in full siblings is consistent with the single locus model. For instance, if $R_M = 50\%$, then R_S must be between 13.2% and 14.2%, whereas if $R_M = 20\%$, then R_S must be between 5.2% and

TABLE 1
Distribution of HLA haplotypes shared IBD for all possible pairs of JDM siblings

	IBD		
	2	1	0
Cudworth and Woodrow[35]	12	10*	2
Barbosa et al.[36]	21	15	2
Spielman et al.[7]	13	16	—
Total	46	41	4

IBD, identical by descent.
* One of Cudworth and Woodrow's sibling pairs was phenotypically identical but scored as IBD = 1 because the parent contributing the A1, B8 haplotype was deceased and may have been homozygous.

6.7%, if the single locus model is to be an adequate hypothesis for the transmission of JDM. Since a wide range of R_M[28,29] and R_S[24,30–35] values have been reported and because of differences between studies in definitions and ascertainment procedures, it is difficult to have confidence in any particular estimates. This is particularly true of some of the older studies where diabetic patients were classified by age of onset rather than by insulin dependence. Consequently it is probable that incidence rates from these early studies included some cases of maturity-onset diabetes of the young. A large-scale, genetic-epidemiologic family study, using strict diagnostic criteria, is necessary before this issue can be fully resolved.

Linkage analysis. Cudworth and Woodrow[36] observed that JDM-affected sibling pairs, when classified according to the number of HLA haplotypes that are inherited IBD, showed a significant perturbation from the a priori expected distribution of ¼, ½, and ¼ for neither, one, or two haplotypes IBD, respectively. Indeed, it was this observation that led them to posit the presence of an HLA-linked JDM susceptibility gene. Table 1 gives the observed distribution of HLA-IBD scores for affected sibling pairs after combining the findings of three separate studies.[7,36,37] There is a significant decrease in the number of sibling pairs sharing neither haplotype IBD (4.4%) and a significant increase in the number of pairs sharing both haplotypes IBD (50.5%).

In order to explore the linkage behavior of JDM under the single locus hypothesis, we fitted Eqs. 6–8 to all consistent values of V_D and V_A shown in Figure 1 (enlargement) by choosing Ψ such that it minimizes the goodness-of-fit chi square (2 degrees of freedom) when tested against the data in Table 1. These results are presented in Table 2 where, for consistent R_M, R_S combinations (in square brackets), the upper figure is the minimum χ^2 for that combination, and the middle figure (in parentheses) is the corresponding θ. Except for R_M, R_S combinations both very small, all con-

TABLE 2
The minimum chi square and its associated recombination fraction, θ (in parenthesis) for a variety of V_D, V_A combinations consistent with the single locus hypothesis obtained by fitting equations 6–8 to the data of Table 1. The figures in square brackets are, respectively, R_M and R_S

	V_A ($\times 10^{-5}$)														
V_D ($\times 10^{-4}$)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
8	—	—	—	—	—	1.94 (15.4) [53,14]	1.94 (15.4) [54,15]	1.93 (14.6) [55,15]	1.91 (14.6) [55,15]	1.89 (14.6) [56,15]	—	—	—	—	—
7	—	—	—	—	1.95 (15.4) [46,12]	1.94 (15.4) [47,13]	1.94 (14.6) [48,13]	1.91 (14.6) [48,13]	1.90 (14.6) [49,14]	1.89 (14.6) [50,14]	—	—	—	—	—
6	—	—	—	1.97 (15.4) [40,10]	1.96 (15.4) [40,11]	1.95 (14.6) [41,11]	1.93 (14.6) [41,11]	1.91 (14.6) [42,12]	1.90 (13.9) [43,12]	1.87 (13.9) [43,12]	—	—	—	—	—
5	—	—	2.00 (15.4) [33, 9]	1.98 (15.4) [33, 9]	1.98 (14.6) [34, 9]	1.94 (14.6) [35,10]	1.93 (14.6) [35,10]	1.90 (13.9) [36,10]	1.87 (13.9) [36,10]	1.85 (13.9) [37,11]	—	—	—	—	—
4	—	—	2.01 (15.4) [26, 7]	2.01 (14.6) [27, 7]	1.97 (14.6) [28, 8]	1.95 (14.6) [28, 8]	1.90 (13.9) [29, 8]	1.88 (13.9) [30, 9]	1.83 (13.3) [30, 9]	1.79 (13.3) [31, 9]	—	—	—	—	—
3	—	2.08 (15.4) [20, 5]	2.06 (15.4) [20, 5]	2.01 (14.6) [21, 6]	1.97 (13.9) [21, 6]	1.93 (13.9) [22, 6]	1.86 (13.3) [23, 7]	1.82 (12.6) [23, 7]	1.75 (12.6) [24, 7]	1.70 (11.9) [25, 8]	—	—	—	—	—
2	—	2.16 (15.4) [13, 4]	2.10 (14.6) [14, 4]	2.03 (13.9) [15, 4]	1.94 (13.3) [15, 5]	1.85 (12.6) [16, 5]	1.75 (11.9) [16, 5]	1.67 (11.2) [17, 5]	1.56 (11.2) [18, 6]	1.46 (10.6) [18, 6]	1.38 (10.0) [19, 6]	—	—	—	—
1	2.51 (15.4) [6, 2]	2.35 (13.9) [7, 2]	2.15 (12.6) [8, 2]	1.94 (11.9) [8, 3]	1.71 (10.6) [9, 3]	1.50 (10.0) [10, 3]	1.31 (9.4) [10, 4]	1.12 (8.8) [11, 4]	0.96 (8.2) [11, 4]	0.81 (7.6) [12, 5]	0.68 (7.6) [13, 5]	0.56 (7.0) [13, 5]	0.47 (6.4) [14, 5]	0.38 (6.4) [15, 6]	—
0.5	3.13 (14.6) [3, 1]	2.58 (11.9) [4, 1]	2.03 (10.0) [5, 2]	1.54 (8.8) [5, 2]	1.12 (7.6) [6, 2]	0.80 (6.4) [6, 3]	0.55 (5.8) [7, 3]	0.37 (5.3) [8, 3]	0.24 (5.3) [8, 3]	0.15 (5.3) [9, 4]	0.09 (4.7) [10, 4]	0.04 (4.7) [10, 4]	0.02 (4.7) [11, 5]	0.01 (4.7) [11, 5]	0.01 (4.7) [12, 5]
0	36.66 (0.5) [0, 0]	4.38 (0.5) [1, 0]	1.39 (0.5) [1, 1]	0.89 (0.5) [2, 1]	0.90 (1.5) [3, 1]	0.90 (2.0) [3, 2]	0.90 (2.0) [4, 2]	0.89 (2.6) [5, 2]	0.90 (2.6) [5, 3]	0.90 (3.1) [6, 3]	0.89 (3.1) [6, 3]	0.89 (3.1) [7, 4]	0.89 (3.1) [8, 4]	0.90 (3.1) [8, 4]	0.91 (3.1) [9, 5]

sistent values in the ranges of interest result in nonsignificant χ^2 for at least some values of the recombination fraction. The higher $R_M - R_S$ pairs, $R_M = 50-60\%$, $R_S = 14-16\%$, support the hypothesis of loose linkage (θ around 15%), whereas lower values (e.g., $R_M = 10\%$, $R_S = 4\%$) support tight linkage ($\theta = 4-8\%$). Intermediate values are appropriately represented in between. The lowest θ values have confidence intervals such that very tight linkage ($\theta < 1\%$) cannot be ruled out. The overall minimum χ^2 is found at $R_M = 13\%$ and $R_S = 6\%$, where θ is between 4 and 5%. For consistent R_M, R_S combinations corresponding to values of V_A that lie along the abscissa of Figure 1 (region A in Figure 2), the χ^2 values are all less than 1.0 and the θ's all smaller than 5%. On the basis of just the results from the linkage analysis, it is impossible to reject the hypothesis that the recombination fraction could be small enough to account for the observed HLA associations via linkage disequilibrium. However, the hypothesis of much looser linkage (θ as high as 15.4%) likewise cannot be rejected. Thus the conclusion about linkage is closely tied to the conclusion about the incidence rates in MZ twins and siblings.

DISCUSSION

In this paper, juvenile diabetes has been treated as a single locus disease in order to gain insight into its behavior vis à vis the joint incidence rates in MZ twins and full siblings and in order to determine if, given the HLA-IBD observations of Table 1, the disease could be tightly linked to the HLA complex.

The mathematical analysis performed here has revealed two important facts concerning the incidence rates R_M and R_S and how they relate to the genetics of JDM: (a) Assuming the population prevalence of JDM to be 1.6/1000, R_M and R_S must fall within the region shown in Figure 2 in order for the single locus model to pertain. High values of R_M (e.g., 50%) require high values of R_S (around 14%) and low values of R_M (10%) require low values of R_S (around 6%). If R_M and R_S are statistically outside this region, then the single locus model must be rejected. (If the actual population prevalence of JDM is higher or lower than assumed here, the results do not change noticeably.) (b) If R_M and R_S are found to lie within the region shown in Figure 2, thus conforming to the single locus model, then we observe a further relationship between the incidence rates and the recombination fraction θ. Based on distortions in HLA haplotype distributions in pairs of affected siblings, the higher $R_M - R_S$ pairs (e.g., 50%, 14%) support the hypothesis of looser linkage ($\theta = 14-15\%$), and the lower pairs (10%, 4%) support tighter linkage ($\theta = 4-8\%$). Very tight linkage ($\theta < 1\%$) cannot be ruled out either. Thus these two pieces of genetic-epidemiologic data, incidences of JDM in the MZ twins and siblings of probands, have the potential of helping us resolve both the genetic mode of transmission of JDM and the question of genetic linkage between JDM and HLA.

It is important to realize that the recombination fractions reported in Table 2 are those that minimize χ^2 *given* a particular set $\{R_M, R_S\}$. The χ^2 values cannot be used to argue that the R_i combinations are correct. The fact that χ^2 approaches zero at $R_M \doteq 13\%$ and $R_S \doteq 5.6\%$ is irrelevant, for instance, if the "true" R_M is eventually found to be 30%. This caution is needed to avoid the fallacy of "confirming" one aspect of the model on the basis of what may prove to be an unwarranted assumption concerning another aspect of the model. For example, Rubinstein et al.[3] treated JDM as an incompletely penetrant recessive disease with a penetrance vector of the form $\{f_1 = f_2 = 0, f_3\}$. This is an assumption concerning the mode of transmission. They then assume that this JDM susceptibility locus is tightly linked to HLA (i.e., $\theta \doteq 0$). This is an assumption concerning the linkage relationship of the two loci. Since only six of twelve HLA-identical siblings of their probands are affected, they deduce that the penetrance of the recessive JDM genotype must be 50% (i.e., $f_3 = 6/12$). But this deduction is a consequence of their assumption of complete linkage, and had they hypothesized that the six HLA-identical, but otherwise unaffected siblings, were unaffected because the putative JDM locus is some distance removed from the HLA complex, their estimate of the penetrance would have been quite different. In other words, some of the unaffected but HLA-identical siblings could be due to recombination and not to incomplete penetrance. The practical implication of this warning is that better data, not the minimum χ^2, are needed to resolve the issues raised here.

Although linkage analysis cannot be used to verify the mode of transmission, it can be useful in detecting heterogeneity once a particular mode of transmission has been hypothesized. Morton,[38] for instance, has shown that elliptocytosis can be caused by at least two different loci, only one of which is linked to the Rh blood groups. Indeed, Morton[39] has argued that tests of heterogeneity may well be considered the raison d'être of linkage detection in man. As a convenient test for heterogeneity, Morton suggests computing the quantity $\mathcal{L} = 4.605(\sum_i \hat{z}_i - \hat{Z})$ where $\hat{z}$ is the maximum lod score for the i^{th} family and $\hat{Z}$ is the maximum of the summed lod scores over all families analyzed. Under large sample theory $\mathcal{L}$ is distributed as χ^2 with $n - 1$ degrees of freedom (n is the number of families studied). We assessed the possible heterogeneity in the data of Barbosa et al.[37] (the only published large set of families where this is possible) under a wide range of transmission hypotheses. In all parameter sets studied so far, there is no hint of heterogeneity, although the technique is insufficiently sensitive to detect a subform if it is quite rare.

Epistasis. Throughout this analysis we have explored the implications of the hypothesis that JDM is determined by a single locus that is linked to the HLA complex. In fact, much of the recent interest in the single locus hypothesis has resulted from the finding of a perturbed sibling pair HLA-IBD distribution and an HLA association in population studies. If it turns out that the single locus model must be rejected, due to incompatibility of R_M and R_S, then we should consider the hypothesis that epistasis between a JDM locus and genes located in or near the HLA complex may be mimicking linkage where none exists. That is, regardless of where the JDM locus is located, if it synergistically interacts with certain HLA antigens, then the JDM phenotype will be correlated with the HLA complex at both the population level (resulting in one or more HLA associations) and the family level (mimicking linkage). Linkage could appear tight or loose depending on the exact mode of transmission of the disease and the nature of its interaction with HLA. This is, of course, a two locus hypothesis similar to one recently advanced for gluten-sensitive enteropathy,[40] which shows an association with the HLA

antigens B8[41] and DW3.[42] In this connection it is interesting to note that when psoriasis vulgaris, which displays a highly significant association with B13 and BW17, is treated as a monogenic disease, linkage to HLA is indicated at a recombination distance of $\theta = 13\%$,[43] a distance too great for linkage disequilibrium to be a likely explanation of the association. Fortunately, the short arm of chromosome 6, where the HLA complex is located, is rich in other known polymorphisms (e.g., GLO, Bf, PGM_3, and C4). If all of these markers are used in a linkage study of multiplex JDM families, it should be possible to decide if an epistatic JDM locus is linked to HLA or is located elsewhere in the genome.

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REFERENCES

[1] Neel, J. V., Fajans, S. S., Conn, J. W., and Davidson, R. T.: Diabetes mellitus. *In* Genetics and the Epidemiology of Chronic Diseases. Public Health Service Publication No. 1163. Neel, J. V., Shaw, M. W., and Schull, W. J., Eds. Washington, U.S. Government Printing Office, 1965, pp. 105–32.

[2] Neel, J. V.: Diabetes mellitus: a geneticist's nightmare. *In* The Genetics of Diabetes Mellitus. Creutzfeldt, W., Köbberling, J., and Neel, J. V., Eds. New York, Springer-Verlag, 1976, pp. 1–11.

[3] Rubinstein, P., Suciu-Foca, N., and Nicholson, J. F.: Genetics of juvenile diabetes mellitus. A recessive gene closely linked to HLA D and with 50 per cent penetrance. N. Engl. J. Med. *297*:1036–40, 1977.

[4] Thomson, G., and Bodmer, W.: The genetic analysis of HLA and disease associations. *In* HLA and Disease. Dausset, J., and Svejgaard, A., Eds. Baltimore, Williams & Wilkins, 1977, pp. 84–93.

[5] Barbosa, J., Chern, M., Noreen, H., Anderson, E., and Yunis, E.: Analysis of linkage between the major histocompatibility system and juvenile, insulin-dependent diabetes in multiplex families: reanalysis of data. J. Clin. Invest. In press.

[6] Suarez, B. K., and Hodge, S. E.: A simple method to detect linkage for rare recessive diseases: an application to juvenile diabetes. Clin. Genet. *15*:126–36, 1979.

[7] Spielman, R. S., Baker, L., and Zmijewski, C.: Genetics of juvenile diabetes: dosage of susceptibility genes. Diabetes *27* (Suppl. 2):459 (Abstract) 1978.

[8] Spielman, R. S., Baker, L., and Zmijewski, C.: Inheritance of susceptibility to juvenile onset diabetes. Paper presented at the Workshop on the Genetics of Common Disease, Snowbird, Utah, August 28–31, 1978.

[9] Svejgaard, A., Platz, P., Ryder, L. P., Staub Nielsen, L., and Thomsen, M.: HL-A and disease associations—a survey. Transplant. Rev. *22*:3–34, 1975.

[10] Nerup, J., Platz, P., Anderson, O., Christy, M., Lyngsøe, J., Poulsen, J., Ryder, L., Nielsen, S., Thomsen, M., and Svejgaard, A.: HL-A antigens in diabetes mellitus. Lancet *2*:864–66, 1974.

[11] Schernthaner, G., Mayr, W., Pacher, M., Ludwig, H., Erd, W., and Eibl, M.: HLA8, W15, and T3 in juvenile onset diabetes mellitus. Horm. Metab. Res. 7:521–22, 1975.

[12] Thomsen, M., Platz, P., Anderson, O., Christy, M., Lyngsøe, J., Nerup, J., Rasmussen, K., Ryder, L., Nielsen, S., and Svejgaard, A.: MLC typing in juvenile diabetes mellitus and idiopathic Addison's disease. Transplant. Rev. *22*:125–47, 1975.

[13] Barbosa, J., Noreen, H., Goetz, F., Simmons, R., Deleiva, A., Najarian, J., and Yunis, E.: Histocompatibility (HLA) antigens and diabetic microangiopathy. Tissue Antigens 7:283–88, 1976.

[14] Nakao, Y., Fukunishi, T., Koide, M., Akasawa, K., Ikeda, M., Yahata, M., and Imura, H.: HLA antigens in Japanese patients with diabetes mellitus. *Diabetes 26*:736–39, 1977.

[15] Rotter, J. I., and Rimoin, D. L.: Heterogeneity in diabetes mellitus—update 1978. Evidence for further genetic heterogeneity with juvenile onset insulin dependent diabetes mellitus. Diabetes *27*:599–608, 1978.

[16] Kempthorne, O.: An Introduction to Genetic Statistics. Ames, Iowa, The Iowa State University Press, 1957, pp. 308–315.

[17] James, J.: Frequency in relatives for an all-or-none trait. Ann. Hum. Genet. *35*:47–49, 1971.

[18] Suarez, B. K., Reich, T., and Trost, J.: Limits of the general two-allele single locus model with incomplete penetrance. Ann. Hum. Genet. *40*:231–44, 1976.

[19] Suarez, B. K.: The affected sib pair IBD distribution for HLA-linked disease susceptibility genes. Tissue Antigens *12*:87–93, 1978.

[20] Suarez, B. K., Rice, J., and Reich, T.: The generalized sib pair IBD distribution: its use in the detection of linkage. Ann. Hum. Genet. *42*:87–94, 1978.

[21] Fishman, P. M., Reich, T., Suarez, B., and James, J.: A note on the essential parameters of the two-allele autosomal locus model. Am. J. Hum. Genet. *30*:283–92, 1978.

[22] Suarez, B. K., Fishman, P. M., and Reich, T.: Estimating the parameters of the incompletely penetrant single locus model using multiple populations. Hum. Hered. *27*:336–51, 1977.

[23] Gorwitz, K., Howen, G., and Thomson, T.: Prevalence of diabetes in Michigan school-age children. Diabetes *25*:122–27, 1976.

[24] Cudworth, A. G.: Type I diabetes mellitus. Diabetologia *14*:281–91, 1978.

[25] Holmgren, G., Samuelson, G., and Hermansson, B.: The prevalence of diabetes mellitus. A study of children and their relatives in a northern Swedish county. Clin. Genet. *5*:465–68, 1974.

[26] Sterky, G.: Diabetic school children. Acta Paediatr. Scand. *144* (Suppl):5–39, 1963.

[27] White, P., and Graham, C.: The child with diabetes. *In* Diabetes Mellitus, 11th edit., Marble, A., White, P., Bradley, R., and Krall, L., Eds. Philadelphia, Lea and Febiger, 1971, pp. 339–60.

[28] Nelson, P. G., Pyke, D. A., Cudworth, A. G., Woodrow, J. C., and Batchelor, J. R.: Histocompatibility antigens in diabetic identical twins. Lancet *2*:193–94, 1975.

[29] Gottlieb, M., and Root, H.: Diabetes mellitus in twins. Diabetes *17*:693–704, 1968.

[30] Köbberling, J.: Genetic heterogeneities within idiopathic diabetes. *In* The Genetics of Diabetes Mellitus, Creutzfeldt, W., Köbberling, J., and Neel, J. V., Eds. New York, Springer-Verlag, 1977, pp. 79–87.

[31] Tattersall, R. B., and Fajans, S. S.: A difference between the inheritance of classical juvenile-onset and maturity onset type diabetes of young people. Diabetes *24*:44–53, 1975.

[32] Nerup, J., Platz, P., Ortved Andersen, O., Christy, M., Egeberg, J., Lyngsøe, J., Poulsen, J. E., Ryder, L. P., Thomsen, M., and Svejgaard, A.: HLA, autoimmunity and insulin-dependent diabetes mellitus. *In* The Genetics of Diabetes Mellitus, Creutzfeldt, W., Köbberling, J., and Neel, J. V., Eds. New York, Springer-Verlag, 1976, pp. 106–114.

[33] Barbosa, J., Chern, M., and Greenberg, L.: Segregation analysis of juvenile-onset, insulin-dependent diabetes (JIDD) in 136 families. Diabetes *27* (Suppl 2):458 (Abstract), 1978.

[34] Harris, H.: The familial distribution of diabetes mellitus: a study of the relatives of 1241 diabetic propositi. Ann. Eugen. (Lond.) *15*:95–110, 1950.

[35] Simpson, N.: Multifactorial inheritance. A possible hypothesis for diabetes. Diabetes *13*:462–71, 1964.

[36] Cudworth, A. G., and Woodrow, J. C.: Evidence for HLA-linked genes in "juvenile" diabetes mellitus. Br. Med. J. *3*:133–35, 1975.

[37] Barbosa, J., King, R., Noreen, H., and Yunis, E. J.: The histocompatibility system in juvenile, insulin-dependent diabetic multiplex kindreds. J. Clin. Invest. *60*:989–98, 1977.

[38] Morton, N. E.: The detection and estimation of linkage between the genes for elliptocytosis and the Rh blood type. Am. J. Hum. Genet. *8*:80–96, 1956.

[39] Morton, N. E.: Segregation and linkage. *In* Methodology in Human Genetics, Burdette, W. J., Ed. New York, Holden-Day, pp. 17–52, 1962.

[40] Peña, A., Mann, D., Hague, N., Heck, J., van Leeuwen, A., van Rood, J., and Strober, W.: Genetic basis of gluten-sensitive enteropathy. Gastroenterology 75:230–35, 1978.

[41] Falchuk, Z., Rogentine, G., and Strober, W.: Predominance of histocompatibility antigen HL-A8 in patients with gluten-sensitive enteropathy. J. Clin. Invest. *51*:1602–06, 1972.

[42] Keuning, J., Peña, A., van Leeuwen, A., van Hooff, J., and van Rood, J.: HLA-DW3 association with coeliac disease. Lancet *1*:506–11, 1976.

[43] Svejgaard, A., Staub Nielsen, L., Svejgaard, E., Kissmeyer Nielsen, F., Hjortshøj, A., and Zachariae, H.: HL-A in psoriasis vulgaris and in pustular psoriasis—population and family studies. Br. J. Dermatol. *91*:145–53, 1974.