Can Future Type I Diabetes Be Predicted?
A Study in Families of Affected Children

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SUMMARY

This article examines the risk of type I (insulin-dependent) diabetes in siblings of affected children, in relation to HLA genotypes. The 288 available siblings of 160 diabetic probands were grouped according to the number of HLA haplotypes in common with their probands. HLA-identical siblings (both haplotypes in common) have an approximately 100 times greater risk of developing the disease than that in the general population, and this risk is significantly higher than that in haplo-identical siblings (one haplotype in common) ($P = 0.008$). Thus, in Northern European populations, some 30% of HLA-identical siblings are expected to be diabetic by the age of 30 yr. The risk in nonidentical siblings (neither haplotype in common) is not significantly increased. These findings carry implications for genetic counseling and research. DIABETES *31*:862–866, October 1982.

It has long been recognized that diabetes shows a familial tendency.[1] Most early attempts to calculate the risk of recurrence (further cases) in the families of affected individuals failed to distinguish clearly between type I (insulin-dependent) and type II (non-insulin-dependent) diabetes, now known to be genetically distinct.[2] Recently, however, Gamble[3] has estimated from the Paediatric Register of the British Diabetic Association that 5.7% of the siblings of diabetic children are themselves affected by age 16 yr.

Hitherto, it has not been possible to predict at an early stage which siblings are destined to become diabetic. In recent years, however, two possible "risk factors" have been identified. One is HLA identity to the diabetic child, the rationale being that genetic susceptibility to this disease is principally HLA-linked.[4] The other is islet-cell antibody (ICA), which is present in around 80% of newly diagnosed diabetic children but only in about 1% of the normal population,[5] in some of whom it may be a "marker" of islet B-cell damage during the prediabetic period.

We have recently reported development of diabetes in seven first-degree relatives of type I diabetic probands while under prospective observation.[6] The presence of ICA in all seven supported the "marker" role of ICA (particularly when complement-fixing, CF-ICA[7]) and provided evidence for a long prediabetic period. Here we analyze further data from the same study to obtain an empirical estimate of risk in the siblings in relation to HLA identity to their probands, based on both new and preexisting cases.

SUBJECTS AND METHODS

Ascertainment. The subjects taking part in the Barts-Windsor Family Study (Table 1) are members of families ascertained through type I diabetic children and young adults attending diabetic clinics in East Berkshire, England, and surrounding districts. Most come from the central district, where the ascertainment rate is probably very high since the great majority of juvenile-onset and most adult-onset insulin-dependent diabetics have at some time attended one or both local diabetic clinics, the records of which are comprehensive and cover the past 20 yr. Criteria for inclusion in the study were availability of the proband and of at least one sibling aged under 20 yr, availability of both parents (or of one parent if there were three or more children), and informed consent. Selection was irrespective of how many children were diabetic. In the central area all but three of the eligible families known to us agreed to take part. Of the families entering the study during its first 2 yr, six were excluded because HLA genotypes revealed expaternity of the proband. All available first-degree relatives (but not half-siblings) of the remaining 160 probands are included in this analysis.

In "multiplex" families (those with more than one diabetic child) the first to have been diagnosed was defined as the proband. All eight such families at the time of ascertainment came from the central, East Berkshire district, with its very

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TABLE 1
Subjects studied in 160 families

	Parents		Probands		Siblings*	
	Fathers	Mothers	Male	Female	Brothers	Sisters
Total number	154	159	86	74	154	134
Age at entry						
Mean (yr)	43.6	40.4	13.8	13.9	14.0	14.6
Range	27–62	26–59	5–24	4–29	1–29	0–32
Number not diabetic	147	157	—	—	152	126

* A further 5 brothers and 10 sisters were unavailable for study. Half-siblings are excluded.

high ascertainment rate. Thus, although this was not planned as an epidemiologic study, there is unlikely to be any significant bias in favor of multiplex families.

Prospective follow-up. Four families were not followed up because all children aged under 20 yr were diabetic. The remaining 156 were reviewed and tested for ICA every 1–4 mo. To facilitate early detection of new cases of diabetes all family members were asked to test for glycosuria at least once monthly using Clinitest or Diastix (Ames, Elkhart, Indiana) after meals. In addition, glycosylated hemoglobin (HbA_1) was assayed at least twice during the study.[8] When a nondiabetic was found to be ICA-positive, by prior agreement this was not disclosed, but random blood glucose and HbA_1 were checked at most visits. If any of these tests suggested hyperglycemia, the diagnosis of diabetes was confirmed or refuted according to WHO criteria.[9]

By late 1980 sixteen families had withdrawn from the full protocol, but occasional contact was maintained for at least 2 yr. For the remaining 140 families the mean follow-up period at the time of this analysis is 2.0 yr (range 1.1–2.5).

HLA typing. HLA genotypes were determined in all families. The standard microlymphocytotoxicity technique was employed, using sera covering all the Caucasoid HLA-A, -B, and -C specificities recognized in 1978. More recently many families have been HLA-DR genotyped, but this work is not yet complete.

Statistical analysis. Except where stated, differences between frequencies are tested for significance using X^2 (with Yates' correction where appropriate). Standard regression techniques are used for trend analyses.[10] The prevalence of diabetes in the siblings is compared with that in the general population using the "relative risk" (RR) or cross-product ratio, with corrections for bias and discontinuity.[11,12] This statistic has the advantage of a readily understood "medical meaning":[11] although more often used as a measure of association between a disease and a blood group or HLA phenotype in population studies, in the present context it is an estimate of the ratio of the risk of diabetes in the study population to that in the general population. It is used here because RR and its 95% confidence limits can be calculated from frequency data in control and study populations, and because the difference between two RR's can readily be tested for significance since $\log_e$ (RR) is approximately normally distributed and its variance is known.

Calculation of prevalence in the general population. There is no entirely appropriate control series of children and young adults without diabetic relatives for comparison with the siblings in this study. We have therefore used published data from two papers reporting occurrence of type I diabetes in the general population. In the first study there were 39 insulin-treated patients among 45,500 school children aged 5–16 yr in Northampton, England, in 1964, giving a prevalence of 0.09% at a mean age of 10.5 yr.[13] This figure was corrected for age by a simple mathematical procedure taking into account both the detailed age distribution of the siblings in the present study and the distribution of age at onset of the disease in published epidemiologic studies[14–17] (combined data by courtesy of Dr. D. R. Gamble).

A further correction was made to compensate for the "frequency reduction effect," that is, the apparent reduction in age-specific cumulative morbidity rate when calculated from prevalence data rather than prospectively from incidence data.[18] The correction factor used (1.1) was obtained by comparing Danish incidence and prevalence figures for the first three decades of life.[14,19] The corrected frequency is 66/45,500, a prevalence of 0.15%.

Similarly, data from an incidence study of insulin-dependent diabetes in Denmark[13] were age-adjusted: over a period of 5 yr 474 new cases were recorded from a population of 716,285 aged 0–29 yr. The frequency expected if this population had the same age distribution as the siblings in the present study would be 1629/716,285, or 0.23%.

RESULTS

Table 2 shows the siblings grouped according to whether they had two, one, or none of the parental HLA haplotypes in common with their probands, these groups being termed HLA-identical, haplo-identical, and nonidentical, respectively. When those with ambiguous assignment to these groups are excluded, the proportions of siblings in the three respective groups are 18%, 56%, and 26%. This distribution differs significantly from that of 25%, 50%, and 25% "expected" on the basis of random assortment of parental haplotypes in children ascertained in this way (see DISCUSSION). In the three cases of intra-HLA recombination so far recognized, siblings are grouped according to B-locus

TABLE 2
Siblings grouped according to HLA-A, -B, -C haplotype concordance with probands

Number of haplotypes in common with proband*	2	2/1†	1	1/0	0	Total
Number of siblings‡	50	8	151	8	71	288
Number diabetic at entry	5	0	4	0	1§	10
(%)	(10)		(3)		(1)	(3)

* Intra-HLA recombinants are grouped according to B-locus identity to proband because not all families have been HLA-DR genotyped (see text).
† 2/1: either two or one haplotype in common with proband, but ambiguous because the two haplotypes in one parent are indistinguishable (10 families).
‡ Distribution of 272 siblings between the three unambiguous groups, compared with the 68, 136, 68 "expected" (see text): $X^2_{(2)} = 6.55$; $P = 0.038$
§ This child has an HLA-B/DR recombinant haplotype and is thus identical to her proband for the DR region of that haplotype.

identity; DR-locus identity would be preferable, but it is not used because DR genotyping is not yet complete.

At the time of entry to the study 9 parents (2.9%) and 10 siblings (3.5%) were already diabetic and insulin dependent. The diabetic siblings were distributed as shown in Table 2; there was no significant sex difference (2 male, 8 female; P = 0.055 by exact binomial test).

During the follow-up period six subjects from these families developed diabetes. Two of these were parents, two were HLA-identical siblings, and two haplo-identical siblings. A further case occurred in an HLA-identical sibling after completion of this analysis and is not included (see DISCUSSION). Individual case reports are given elsewhere.[6]

Current prevalence of diabetes and relative risk in siblings. At the time of this analysis, 11 (3.5%) of the parents and 14 (5%) of the siblings have type I diabetes. The prevalence in the siblings (grouped according to HLA-ABC genotype identity to their probands) is shown in the upper section of Table 3. Also given in this table are the relative risks for diabetes calculated by comparing the frequencies in the siblings with the age-adjusted frequencies derived from the Northampton study (middle section of Table 3) and the Copenhagen study (bottom section) as low and high estimates of general population frequency. Whichever set of control figures is used, the RR for HLA-identical siblings is significantly higher than that for haplo-identical siblings (P = 0.011 and 0.008, respectively; one-tailed test assuming normal distribution of $\log_e$RR). In the case of nonidentical siblings, only one of whom is diabetic, the Woolf-Haldane method is inappropriate, but the binomial test shows that the frequency of diabetes is not significantly greater than that in controls.

DISCUSSION

These findings provide further support for the well-established concept that genetic susceptibility to this disease is mainly HLA-linked, in that the prevalence in the siblings is significantly associated with increasing HLA haplotype concordance with probands (Table 3). This point is underlined by the finding that the only nonidentical sibling who is diabetic has a B-DR recombinant haplotype, so that if DR-locus identity were the criterion used (as it would be if all families had been DR-typed), even she would be classed as haplo-identical.

TABLE 3
Current prevalence of diabetes in siblings grouped by HLA genotype identity to probands

Group	HLA-identical	Haplo-identical	Non-identical	All siblings*
Number of siblings	50	151	71	288
Number diabetic	7	6	1	14†
Prevalence (%)	14	4	1	5
Compared with N:‡ relative risk (95% confidence limits)	118 (52–265)	31 (13–69)	NS	36 (20–64)
P	$<10^{-10}$	$<10^{-10}$	0.09§	$<10^{-10}$
Compared with D:‡ relative risk (95% confidence limits)	76 (35–164)	20 (9–43)	NS	23 (14–39)
P	$<10^{-10}$	$<10^{-10}$	0.15§	$<10^{-10}$

* Including those with ambiguous HLA genotypes (see text).
† Distribution among nonambiguous groups compared with that expected if no linkage between HLA and diabetes: P = 0.0053. Analysis for quadratic trend: P < 0.005.
‡ Age-corrected frequencies of diabetes in general population samples (see text): N, Northampton, 1964:[13] 66/45,500 (0.15%); D, Denmark, 1977:[14] 1629/716,285 (0.23%).
§ One-tailed binomial test.

The second and major point of this article is the risk of diabetes in siblings of affected children. An empirical approach to this problem, taking into account HLA haplotype concordance, is important. Others have used indirect calculations based on haplotype concordance frequencies observed in pairs of siblings selected because both members of each pair were diabetic.[20,21] They assume that 25%, 50%, and 25% of all siblings of diabetic children are, respectively, HLA-identical, haplo-identical, and nonidentical to them, these being the proportions predicted by Mendelian rules in sibships randomly ascertained with respect to HLA.

However, the present series, which is believed to be the largest of its kind, shows a significant difference from that distribution, with only 18% in the HLA-identical group (Table 2). This difference is not due to errors in HLA typing, which was repeated in case of any doubt; in fact, the majority of families have now been typed at least twice without any evidence of incorrect assignment to haplotype concordance groups on the first occasion. Furthermore, serologic problems with HLA typing should not lead to a systematic error of this kind. Nor can the discrepancy be explained by biased selection of families (see SUBJECTS AND METHODS).

We believe the true explanation to be as follows. Although the proband-sibling pairs in this (or any other) sample come from a population of families in which HLA haplotype segregation is (presumably) random, the selection process necessarily introduces a bias. This is a consequence of the fact that the probands have an HLA-linked disease, and can be predicted using simple probability,[22] as illustrated in the following example.

For simplicity, let us consider first a large, hypothetical series of sibships, each consisting of two healthy children. We assume that within each sibship there is random assortment of the four possible haplotype combinations. If one child from each sibship were chosen arbitrarily as the index case, then the proportions of all siblings of these index cases falling into the three haplotype-concordance classes would indeed be close to 25%, 50%, and 25%. Consider now, for the sake of argument, a series of similar families selected for presence of a specific HLA allele (Bw44, for example) in one paternal and one maternal haplotype; 25% of the children would be Bw44-homozygous, but 56.25% [i.e., $(1 - 0.25)^2 \times 100$] of the families would have no such child, while 6.25% would have two. If, now, families were ascertained from this series through Bw44-homozygous children (the probands) to a genetic study, 43.75% of all the families would be included. Since each proband has one sibling, and only 6.25% of the original series of families include two homozygotes, the expectation is that 6.25 × 100/43.75 = 14.29% of the siblings of the probands in the study would be HLA-identical to them—not the 25% that might have been anticipated.

A similar argument can be applied to diabetes:[22] because of the combination of ascertainment of families through affected children and HLA-linkage of the disease, the proportion of siblings expected to be HLA-identical to the pro-

bands is less than 25%. The extent of this bias depends on the penetrance of the susceptibility genes (which, of course, is related to age and other factors), their mode of inheritance, the closeness of linkage to HLA, and family size.

Using the indirect method referred to above, Platz and co-workers[21] calculated that the prevalence expected in HLA-identical, haplo-identical, and nonidentical siblings in Denmark at age 16 yr would be about 12%, 4%, and 1%, respectively. Despite the assumption of HLA-identity in 25% of siblings, the similarity of these estimates to the prevalences actually observed here (Table 3) is striking, even though they are not strictly comparable because the populations concerned have different age distributions.

There is in theory a possibility of bias due to more assiduous screening for diabetes in our families than in the general population. In fact all but one of the 14 diabetic siblings developed characteristic symptoms and would undoubtedly have been diagnosed in any event. The single exception, who is HLA-identical to her proband, is not yet insulin dependent; but not included in this analysis is a seventh child, also HLA-identical to his proband, who has since developed symptomatic type I diabetes. Therefore, inclusion of the asymptomatic case is unlikely to cause any significant bias.

For the calculation of RR in the siblings in our study, it is not clear whether the estimate of general population frequency based on the Northampton data or that from the Copenhagen data is the more appropriate. Both have therefore been used. Gamble has argued that the Danish figures can be applied to the United Kingdom, and has estimated by a different method that the overall risk in siblings of diabetic children at age 16 yr is 27 times greater than that in the general population.[3] This is close to our estimate of 23 using the Copenhagen data, but is also well within the 95% confidence limits of that using the Northhampton data (Table 3). Whichever "control" figures are used, when siblings are grouped according to HLA haplotype concordance with the probands (Table 3), two features stand out. The RR for HLA-identical siblings is extremely high, and it is considerably and significantly higher than that for haplo-identical siblings. These results reinforce other evidence that HLA-linked susceptibility to type I diabetes is not determined by a single, dominant gene, and are compatible with theories involving recessive or intermediate inheritance or interaction of two or more genes.[4,21,23,24]

Finally, we return to the question of predicting recurrence of this disease in particular sibships. It seems reasonable to suggest that for each group of siblings the mean of the RRs calculated from the two sets of control data is a fair approximation to the true value. In that case, if HLA genotypes are disregarded, a sibling of an affected child carries approximately a 30-fold increased risk, and if the two are HLA-identical, the risk is increased around 100-fold. Thus some 30% of these siblings would be diabetic by age 30 yr, in Northern Europe, assuming that the general population prevalence at that age is 3 per 1000 (a conservative estimate from the Northampton study and from the combined age-at-onset data,[14–17] similar to the prevalence observed in Denmark[19]).

The finding of CF-ICA also increases the risk. That CF-ICA-positive nondiabetic subjects may later develop type I diabetes has been reported in a few instances, but many of these were adults with other autoimmune endocrine conditions,[25,26] whereas the remainder were single cases,[27] including one of six CF-ICA-positive siblings in a smaller family study. In the present prospective study, as already reported,[6] each of the six who later developed diabetes was already positive for conventional ICA when first tested, so that 11% of the 54 conventional-ICA-positive nondiabetic subjects have become diabetic compared with 0% of those who remained ICA-negative. This difference is highly significant ($2P = 10^{-6}$, Fisher's exact test). The equivalent figures for CF-ICA, which was detected in 18 initially nondiabetic subjects, are 28% and 0.2%, respectively ($2P < 10^{-6}$). When these data are expressed in risk terms, the Woolf-Haldane method gives an RR of 73 (95% confidence limits 11–483) for development of diabetes in the initially healthy parents and siblings who were CF-ICA positive, compared with those who remained CF-ICA negative throughout the follow-up period. This RR should be interpreted with caution because new cases appearing while under review are as yet very few for this type of analysis, and because only a restricted period in the life of each participant has been surveyed. Nevertheless, it is clear that the combination of a positive CF-ICA result and HLA-identity to a diabetic sibling must give a child a very high risk of diabetes, even though the combined risk cannot as yet be quantified accurately.

In conclusion, we believe this to be the first study providing sufficient HLA and (prospective) ICA data for estimation of empirical risk of developing type I diabetes in different groups of siblings. HLA genotyping proves to be a powerful tool enabling the definition of one group of siblings having a very high risk of diabetes, and another with a negligible risk either of developing the disease or, presumably, of transmitting genetic susceptibility to future generations. This has considerable importance for genetic counseling.

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