Evidence That the Age at Diagnosis of IDDM Is Genetically Determined

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OBJECTIVE — The aim of this study was to determine the impact of genetic or environmental factors on the age or time of onset of IDDM by studying pairs of twins and siblings concordant for the disease.

RESEARCH DESIGN AND METHODS — From 404 twin pairs referred to a diabetic twin study, we selected pairs concordant for IDDM: *1*) 116 identical pairs with an index twin diagnosed diabetic under age 60 years and 2) 12 identical and 12 nonidentical matched twin pairs. From 972 families referred to a population-based diabetic family study, we selected sibling pairs with IDDM: 33 pairs with an index case diagnosed diabetic under age 21 years. Twin and sibling pairs were analyzed for intraclass correlations for age and time of diagnosis.

RESULTS — Of twins concordant for IDDM, the age at diagnosis correlated *1*) in 116 identical pairs ($R = 0.94$; $P < 0.000001$) and 2) more closely in 12 identical twins ($R = 0.96$, $P < 0.000001$) than 12 nonidentical twins ($R = 0.59$, $P = 0.046$). Of 33 sibling pairs with IDDM, the age, but not the time, of diagnosis was correlated ($R = 0.53$, $P = 0.0016$).

CONCLUSIONS — Correlations within pairs of twins and siblings for age, not time, at diagnosis suggest that much of the variability of the age at diagnosis of IDDM is genetically determined.

IDDM is caused by an interaction of genetic and environmental factors. It is likely that the destructive immune process is induced by environmental factors operating in early life (1). Thereafter, there is a prolonged disease incubation period during which both cellular and humoral immune changes can be detected in peripheral blood (2,3). Because the age at clinical onset of IDDM varies widely, the incubation period is also likely to vary. We do not know to what extent genetic or environmental factors determine the variable rate of disease progression.

Recent population studies have demonstrated space-time clustering for IDDM in children consistent with a common exposure to local environmental factors (4,5). Two lines of evidence suggest that genetic factors may also be involved in determining the age of onset of IDDM. First, IDDM patients diagnosed in childhood are more likely to have those HLA genes associated with disease susceptibility, such as HLA DR3/4 and HLA-DQA1*0301-DQB1*0302, than adults (6). Second, identical twins concordant for IDDM tend to be diagnosed within a few years of each other, and the risk of developing diabetes in the co-twin declines with time from the disease onset in the index twin (7). These two observations raise the possibility that genetic factors are important in determining the age of onset of IDDM and, by implication, the disease incubation period.

The aim of this study was to determine the impact of genetic and environmental factors on the age of onset of IDDM. If shared factors were important, we would expect a significant correlation between identical twins for age at diagnosis. A genetic effect would be reflected in a closer age at diagnosis in identical compared with nonidentical twins. A shared environmental exposure should lead to a similar time of diagnosis. Because twins are the same age, it is not possible to determine whether similarities between them relate to their age or to their time of diagnosis. We, therefore, studied pairs of affected siblings as well as twins to determine whether the age or the time of diagnosis in the index sibling correlated with that of the second affected sibling.

RESEARCH DESIGN AND METHODS

Twins

IDDM was defined according to the National Diabetes Data Group criteria (8). Age and time of diagnosis were documented to the nearest month by the referring physician and then checked in an interview with the patient. Six pairs in which the age at diagnosis of a twin could not be determined to the nearest year were excluded. Age at onset of IDDM was taken as time of diagnosis and not as time of starting insulin treatment, although in >95% of cases this was the same.

We studied twin pairs from the British Diabetic Twin Study referred between January 1966 and June 1996 because they had diabetes, not because they were twins. Monozygosity between twins was established as previously described (2,7). We selected two sets of pairs concordant for IDDM from 404 pairs referred to the study: *1*) all identical twins referred to the study in whom the index twin was diagnosed with IDDM under the age of 60 years and who are now concordant for IDDM (to limit ascertainment bias due to pairs diagnosed at a similar age being referred to our study, we also considered a subgroup of pairs referred within 2 years of diagnosis of the index twin, which became concordant under observation) and 2) pairs of nonidentical twins concordant for IDDM compared with identical twins concordant for IDDM who were selected because the index twins were matched for sex and age at diagnosis (both groups were ascertained in the same way and referred to the study because the index case was a twin with IDDM).

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Abbreviations: ΔAOD, difference in age of diagnosis; ΔDOB, difference in date of birth; ΔDOD, difference in date of diagnosis.

The twin groups were composed of two sets of individuals. The first set consisted of 116 identical twin pairs (55 male) concordant for IDDM for which the mean ± SD age of diagnosis of the index diabetic twin was 16.2 ± 12.9 years (range 0.3–59.0), and that of the second twin was 21.3 ± 14.8 years (1.6–64.7). Of these twins, 19 pairs became concordant under observation; the mean age of diagnosis of the 19 (11 male) index twins was 10.4 ± 4.4 years (2.0–18.5), and that of the second twin was 13.0 ± 5.5 years (2.7–23.5). These 19 pairs were part of a cohort of 49 pairs followed prospectively. Of the 30 twin pairs (15 male) who remain discordant for IDDM, the mean follow-up from the diagnosis of the index twin was 19.4 ± 6.2 years (7.0–30.7); all 30 twins had normal glucose tolerance and were without islet cell antibodies (<5 Juvenile Diabetes Foundation U), with an estimated disease risk of <2%. Thus, the chance that further twins from this cohort of 49 pairs will develop IDDM later, and thereby alter the correlations, is very small. The second set consisted of 12 identical twin pairs (7 male) concordant for IDDM who had a mean age of diagnosis of the index twin of 12.4 ± 6.0 years (5.5–22.4) and of the second twin of 15.3 ± 6.7 years (7.6–26.5). These 12 index identical twins were matched with 12 index nonidentical twins (7 male) from pairs concordant for IDDM with a mean age of diagnosis of the index twin of 11.9 ± 6.3 years (5.5–22.4) and of the second twin of 20.5 ± 8.7 years (7.4–36).

Siblings

Sibling pairs concordant for IDDM were selected from 1,542 siblings from 972 families in the Bart's Oxford Family Study, which is population-based, and entered between 1985 and June 1996 (9,10). Probands with IDDM were reported to the coordinating center by local medical practitioners and diabetes nurse specialists because they were residents in the region of study and <21 years of age at diagnosis with IDDM as previously defined (10). The ascertainment is >95%. We excluded from our analysis *1*) all twin pairs, since we were comparing twin and siblings data; 2) sibling pairs in which the second sibling was not dependent on insulin from diagnosis; 3) sibling pairs in which one sibling was born after the other sibling had developed IDDM, since it is not possible to compare such siblings to determine the effect of shared environmental factors; and 4) individuals who already had two siblings included in the study. The date of diagnosis was defined as the day insulin was started and checked in an interview with the patient. Data were assessed for all eligible sibling pairs. In addition, we studied those sibling pairs born apart by more than the mean number of years difference between all the sibling pairs. This latter group is likely to be more informative because it reduces clustering in affected siblings as a result of a tendency for siblings in a family to be born within a few years of each other and a tendency for IDDM to be more prevalent at age 5 years and during puberty (9,10).

The pairs that fulfilled the ascertainment criteria comprised 33 sibling pairs (44 male, 22 female) concordant for IDDM for which the mean ± SD age of diagnosis of the index diabetic sibling was 11.3 ± 5.5 years (2.4–20.0), and that of the second sibling was 10.7 ± 6.5 years (1.3–23.9). Of these, 11 sibling pairs were born apart by more than the mean difference in age for all affected sibling pairs, that is, >3.7 years apart. The mean age of diagnosis of the index diabetic sibling in this second group was 10.3 ± 4.7 years (2.4–16.6), and that of the second sibling was 10.9 ± 6.5 years (2.7–19.5).

Statistics

Results are presented as means ± SD or medians (range) when the values were nonparametrically distributed. $P < 0.05$ was taken as statistically significant.

The intraclass correlation (R) and coefficient of determination (R^2) between age at diagnosis was estimated using simple linear correlation coefficients for groups of identical and nonidentical twin pairs to test whether they had a similar age of onset of IDDM. To determine whether age of diagnosis of the index twin influenced the correlation between twins, we also tested the linear correlation between age at diagnosis of the index twin and the differences in ages of diagnosis between twins of each pair.

To test whether genetic factors influence age at diagnosis, we compared identical and nonidentical twin pairs. Identical twins share identical genotypes, so differences between them, theoretically, are due to environmental factors. Nonidentical twins, in contrast, only share 50% on average of their segregating genes. Thus, the extent to which identical twins are more alike for age at diagnosis than matched nonidentical twins reflects genetic influences and can be tested by *1*) comparing differences for age at diagnosis between twins of each pair using Student's t test for paired samples and 2) comparing intraclass correlations between twins; in the classic twin method, the difference between intraclass correlations for identical and nonidentical twins is doubled to estimate heritability.

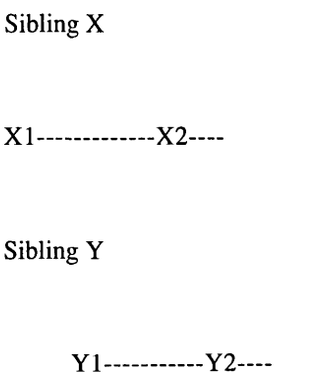

Figure 1—*For two siblings (X and Y), X1 and Y1 are their dates of birth and X2 and Y2 are their dates of diagnosis. ΔDOB is X1 − Y1. When ΔDOB = ΔDOD, the age at diagnosis is the same, and when ΔDOB = ΔAOD, the date at diagnosis is the same. Thus, a correlation between ΔDOB and ΔDOD indicates a similar age at diagnosis, while a correlation between ΔDOB and ΔAOD indicates a similar date of diagnosis (11).*

We tested the correlations for age or date of diagnosis for all eligible sibling pairs and sibling pairs born >3.7 years apart (the mean difference between all the sibling pairs). To avoid bias due to birth order, the tendency within families for siblings to be born within a few years of each other, and the tendency for patients with diabetes to have similar ages at diagnosis, we analyzed sibling data differently from twin data (11). Correlations were determined by simple linear regression between differences in date of birth (ΔDOB) and differences in either their age of diagnosis (ΔAOD) or their date of diagnosis (ΔDOD) (Fig. 1) (11). When ΔDOB = ΔDOD, the age at diagnosis is the same, and when ΔDOB = ΔAOD, the date of diagnosis is the same. Thus, in siblings, a correlation for ΔDOD with ΔDOB and a mean ΔAOD approximating zero indicates a similar age at diagnosis, while a correlation for ΔAOD with ΔDOB and a mean ΔDOD approximating zero indicates a similar date of diagnosis (Fig. 1). A similar analysis could not be performed on twins because these variables are equal (i.e., ΔAOD = ΔDOD) and ΔDOB = 0.

The subjects or their parents gave informed consent, and the study was

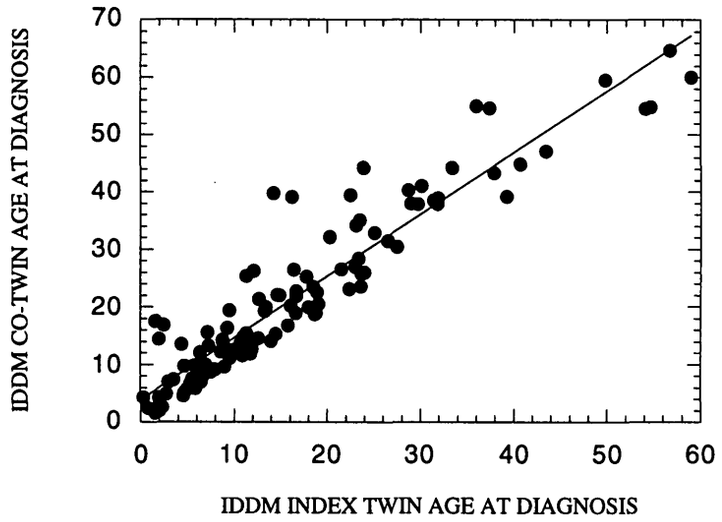

Figure 2—*Age at diagnosis in 116 identical twin pairs concordant for IDDM. There was a strong correlation between twins (R = 0.94).*

approved by the ethical committee at St. Bartholomew's Hospital.

RESULTS

Twins

The 116 identical twin pairs concordant for IDDM had a correlation coefficient for age at diagnosis of 0.94 (R^2 = 0.88, P < 0.000001) (Fig. 2). There was a similar correlation in male twin pairs (R = 0.93) and female twin pairs (R = 0.96). The difference in age at diagnosis between identical twins (median difference 3.6 years [range 0.01–25.7]) was weakly related to the age at diagnosis of the index twin, so that the difference increased as the age of diagnosis of the index twin increased (R = 0.19, R^2 = 0.034, P = 0.047). However, the striking correlation between identical twins was evident throughout the age range (Fig. 2), and the correlation in twin pairs for age at diagnosis was similar irrespective of whether the index twin was more or less than the mean age (16.2 years) (R = 0.93 and 0.71, respectively).

The 19 identical twin pairs who became concordant under observation showed a strong correlation between twins for their age at diagnosis of IDDM (R = 0.95, R^2 = 0.89, P < 0.000001). None of the 30 twins remaining nondiabetic at ≥7 years had altered glucose tolerance or islet cell antibodies, so their estimated disease risk is now <2% (2).

The correlation for age at diagnosis between pairs in the group of 12 identical twins concordant for IDDM (R = 0.96, R^2 = 0.93, P < 0.000001) was greater than that in the matched set of 12 nonidentical twins concordant for IDDM, though they also showed a significant correlation (R = 0.59, R^2 = 0.3, P = 0.046) (Fig. 3). Estimated heritability for age at diagnosis was 74%, consistent with a genetic influence on age or time of diagnosis. The difference in age at diagnosis between identical twins (mean difference 2.8 ± 1.9 years) was significantly less than that for nonidentical twins (mean difference 8.5 ± 7.2 years) (P = 0.015).

Siblings

The 33 sibling pairs showed a significant correlation for their age at diagnosis of IDDM, i.e., ΔDOB correlated with ΔDOD (R = 0.53, R^2 = 0.28, P = 0.0016), and the mean ΔAOD approximated zero (ΔAOD = 0.55 ± 7.03 years). There was no correlation for the date of diagnosis, i.e., ΔDOB did not correlate with ΔAOD (R = 0.24, R^2 = 0.06, P = 0.17), and the mean ΔDOD did not approximate zero (ΔDOD = 3.14 ± 8.34 years).

Sibling pairs (n = 11) who were not of a similar age (>3.7 years difference) had similar results. In these pairs, there was also a significant correlation for age at diagnosis of IDDM, i.e., ΔDOB correlated with ΔDOD (R = 0.65, R^2 = 0.42, P = 0.03), but not for the date of diagnosis, i.e., ΔDOB did not correlate with ΔAOD (R = 0.42, R^2 = 0.17, P = 0.20).

CONCLUSIONS — The analysis of 116 identical twin pairs concordant for IDDM revealed a striking correlation between twins for their age at diagnosis consistent with shared genetic and environmental factors having a major effect on the time of diagnosis of the disease. To confirm and extend this observation, we eliminated potential sources of error by performing further analyses on selected identical twins and siblings. Biased referral might result in the ascertainment of twins or siblings diagnosed at a similar age or time. We, therefore, studied identical twin pairs who had been referred as discordant for IDDM who became concordant under

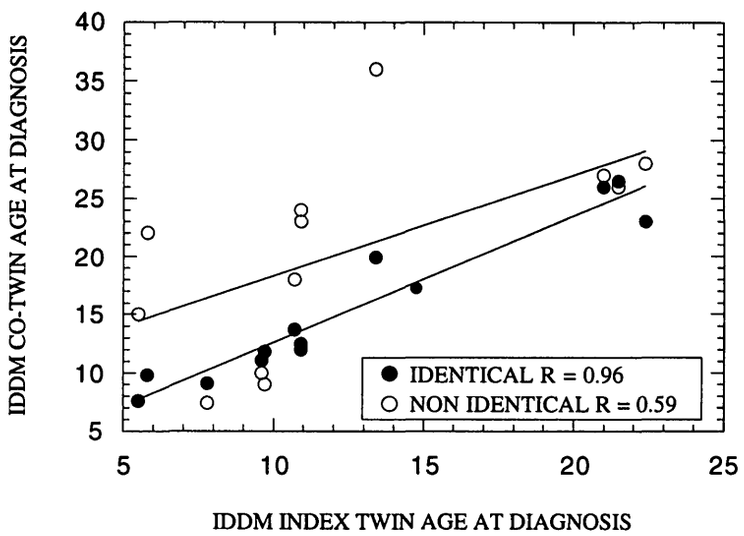

Figure 3—*Age at diagnosis in 12 identical and 12 nonidentical twin pairs concordant for IDDM. There was a stronger correlation between identical than between nonidentical twins (R = 0.96 and 0.59, respectively). Estimated heritability of age at diagnosis is 74%.*

observation, and we identified affected sibling pairs from a population-based study. In addition, insufficient follow-up could result in apparent clustering by not detecting twins who will become diabetic at a later stage; we, therefore, followed the twins who remained nondiabetic prospectively for at least 7 years, and in our cohort, the estimated risk of developing diabetes was very low in those twins who remained not diabetic (2). Finally, to avoid bias due to birth order, the tendency within families for siblings to be born within a few years of each other, and the tendency for patients with diabetes to have similar ages at diagnosis, we sought correlations in siblings between the differences in their dates of birth and differences in either their age of diagnosis or their date of diagnosis (11,12).

The selected cohort of identical twin pairs who became concordant for IDDM under observation confirmed a strong correlation for their age of onset of IDDM. To evaluate the relative contribution of shared genetic or environmental factors with the age of diagnosis of IDDM, we then compared a small number of identical and nonidentical twin pairs concordant for the disease. The index twins in both groups were matched for age at diagnosis and sex, and the method of ascertainment was similar. Both identical and nonidentical twins showed a significant correlation for age of onset of IDDM, but it was substantially greater in identical twins (0.96 vs. 0.59), giving an estimated heritability of 74%. In line with a genetic influence on the age at diagnosis, there was a smaller variation for age at diagnosis in identical than in nonidentical twins. These observations indicate that genetic factors strongly affect the clinical onset of IDDM and, by implication, the rate of progression of the destructive process during the prediabetic period.

To resolve whether the correlation between twins for age at diagnosis could be related to the age or the time of diagnosis, we studied families with two affected siblings, since siblings, unlike twins, are born at different times. We found a correlation between siblings for age, but not for time, of diagnosis. This observation is consistent with two previous studies of 69 and 26 multiplex families, respectively, which also showed a significant correlation between siblings for their age of IDDM onset (12,13). The striking correlation between identical twins for age at diagnosis of IDDM implies that the period from disease induction to diagnosis of diabetes is similar. Our observations suggest that this correlation between identical twins may not be entirely genetically determined and that the environmental effect on age at diagnosis increases with increasing age of onset in the index twin, if only slightly. This environmental effect could operate either to induce the disease process or to precipitate clinical onset. We have previously speculated that an environmental factor could induce the disease process during a period of susceptibility in early life (1). It is unlikely that a common environmental effect precipitates the clinical onset, since there was no correlation in siblings for the date of diagnosis. It is possible, however, that pairs of siblings tend to develop clinical diabetes at a similar age because of a common effect, such as puberty.

While our data indicate that genetic factors play a role in the age of diagnosis of IDDM, they did not identify whether non-HLA, as well as HLA, genes could be relevant in determining the time of diagnosis. HLA genes are associated with disease susceptibility and some genotypes, such as HLA DR3/4 and HLA-DQA1*0301-DQB1*0302, are found more often in young IDDM patients than in those diagnosed in adult life (6). The present results raise the possibility that the IDDM disease process is more aggressive in subjects with certain HLA, and possibly non-HLA, genes (14). Interestingly, in distinct transgenic mouse models in which viruses induce IDDM, major histocompatablity complex genes were found to play a modulating role in determining the rate of progression of the disease process (15). Recent studies in human immunodeficiency virus disease have also implicated certain HLA types in rapid or slow progression of the clinical disease and the loss of CD4 lymphocytes (16). To determine the role of HLA or non-HLA genes in determining correlations between siblings for their age at diagnosis will require a study of HLA identical and nonidentical sibling pairs. The number of affected sibling pairs in our population-based family study is insufficient to perform such an analysis at present.

Autoimmune diseases are caused by an interaction of genetic and environmental factors. Genetic factors could operate in a number of ways to influence the risk of disease or the demographic characteristics of the disease. Our present study suggests that genetic factors may also influence the rate of disease progression and, hence, the time at which clinical diabetes presents. Age at onset in identical twins is correlated in other autoimmune diseases, including multiple sclerosis, systemic lupus erythematosus, and rheumatoid arthritis (11,17,18). Family studies in multiple sclerosis suggest that in that disease the correlation is also with age and not time of diagnosis (11,19). It is likely that autoimmune diseases involve induction of an immune process by an environmental event (1). Our present studies indicate that the subsequent variation in the disease incubation period, at least for IDDM, is strongly genetically influenced.

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