Concordance for Type 1 Diabetes in Identical Twins Is Affected by Insulin Genotype

Karl A. Metcalfe, MRCP
Graham A. Hitman, MD
Rachel E. Rowe, MD
Mohammed Hawa, BSc

Xiaojian Huang, PhD
Timothy Stewart, PhD
R. David G. Leslie, MD

OBJECTIVE — Monozygotic twins are usually discordant (only one twin affected) for type 1 diabetes. Discordance for disease between such twins implies a role for nongenetically determined factors but could also be influenced by a decreased load of diabetes susceptibility genes. The aim of this study was to determine whether two susceptibility genes were less prevalent in discordant twins compared with concordant twins.

RESEARCH DESIGN AND METHODS — We studied 77 monozygotic twin pairs (INS), 40 concordant and 37 discordant, for type 1 diabetes at polymorphism of the insulin gene region on chromosome 11p and HLA-DQB1.

RESULTS — The disease-associated INS genotype (Hph I) was identified in 87.5% of the concordant twins but only in 59.5% (P = 0.005) of the discordant twins. Neither DQB1*0201 nor DQB1*0302 was seen in 2 of 40 (5%) concordant twins compared with 8 of 37 (22%) discordant twins (P = 0.04). No statistical differences were seen between concordant and discordant twins at individual alleles of DQB1. Combining insulin and DQ data, 5% of concordant twins compared with 32.4% of discordant twins had neither DQB1*0201/DQB1*0302 nor the high-risk Hph I INS “++” genotype (P = 0.002).

CONCLUSIONS — We conclude that the possession of the high-risk Hph I insulin genotype increases the likelihood of identical twins being concordant for type 1 diabetes and that the “load” of both major histocompatibility complex (MHC) and non-MHC susceptibility genes has an impact on the disease penetrance of type 1 diabetes.

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Concordance rates for type 1 diabetes are higher in monozygotic than dizygotic twins, which is consistent with a role for genetic factors determining the disease (1). Nevertheless, ∼50% of monozygotic twins are discordant for type 1 diabetes, providing strong evidence that nongenetically determined factors also influence susceptibility to disease.

At least 30% of the genetic susceptibility to type 1 diabetes can be explained by an association with the major histocompatibility complex (MHC) (2). Although a large number of non-MHC chromosomal regions have been implicated in disease risk, only a few with known function have been identified, including the insulin gene hypervariable region (2–5), the interleukin gene cluster (6,7), CTLA4 (8–10), and the vitamin D receptor (11,12). However, the only consistent association between type 1 diabetes and a non-MHC region is with the insulin gene.

The study of monozygotic twins concordant or discordant for type 1 diabetes is a potentially powerful method to determine whether disease penetrance is a random effect or influenced by genetic factors. Monozygotic twins tend to develop type 1 diabetes within 6 years of each other (13), so that twin pairs discordant for diabetes are likely to remain discordant. We identified such discordant twin pairs in a previous HLA phenotype study and showed that they were less likely than concordant pairs to have both HLA-DR3 and HLA-DR4 antigens (14). Subsequent studies indicated that pairs remaining discordant are more accurately defined as nondiabetic twins more than 11 years from the diagnosis of the index twin, with normal glucose tolerance and without antibody markers (15–17). Because there are no previous genotype studies of HLA-DQB1 or non-MHC-encoded susceptibility genes to type 1 diabetes in identical twins, we have now tested discordant as well as concordant pairs using this definition of discordance. Our hypothesis was that discordance, as compared with concordance, for type 1 diabetes in monozygotic twins is caused by a decreased load of both MHC and non-MHC susceptibility genes.

RESEARCH DESIGN AND METHODS — We have studied and compared 40 concordant and 37 discordant monozygotic twins for type 1 diabetes at both HLA-DQB1 and the insulin gene (INS) loci. Twins studied were selected blind to those included in our previous study 15 years ago of HLA-DR phenotype (14). A total of 54% of twin pairs were common to both studies.

Twins were ascertained from 1967 to
1997 by referral to the British Diabetic Twin Study; monozygosity was established in each twin pair as described previously (18,19). DNA was not available from all twins. All twins were invited to attend the Diabetes Department for blood sampling, but in the study period, only a fraction were able to do so. We therefore selected twin pairs who had attended the Diabetes Department consecutively from 1995 to 1997, who were either concordant or discordant for type 1 diabetes. The diabetic index twin from both concordant and discordant pairs had a comparable age at diagnosis, and nondiabetic twins from discordant pairs had a low risk of developing diabetes. Discordant pairs likely to remain discordant were identified by being >11 years from the diagnosis of the index twin and, at 11 years by having normal glucose tolerance and the absence of islet cell antibody (ICA), GAD antibody, or IA-2ic antibody.

Of 247 discordant twin pairs, we selected a consecutive series of 40 pairs attending the Diabetes Center (mean age of diagnosis of index twin 16 ± 9 years, range 1–39; 16 male pairs; median time to concordance 3.95 years, range 0.4–25). Of 109 discordant twin pairs, we identified a consecutive series of 37 pairs (mean age of diagnosis of index twin 19 ± 11 years, range 2–59; 20 male pairs) in which the index twin had a comparable age at diagnosis to the index twin of the discordant pairs. Nondiabetic twins underwent oral glucose tolerance testing (glucose was given as 75 g or 1.75 g/kg, whichever was less) to confirm that they had neither diabetes nor impaired glucose tolerance both initially and at intervals thereafter; diagnosis of type 1 diabetes was made according to standard guidelines (20). Sera were stored at −20°C and analyzed for each of three antibody assays. Analysis was performed on batched samples by observers blinded to the clinical status of the subjects.

**Autoantibody assays**

IA-2ic and GAD$_{65}$ were measured by radioimmunoprecipitation assays as described previously (Promega, Madison, WI) (17). All samples were tested in duplicate, including positive and negative control standard sera. Each assay for IA-2ic and GAD$_{65}$ antibodies included serially diluted sera from a patient with stiff-man syndrome and a prediabetic individual (a twin who subsequently developed type 1 diabetes but was not part of this study) to further evaluate the cutoff level for positivity for GAD$_{65}$ and IA-2ic. Levels >3 SD higher than that of a control population were considered positive, as described previously (17). In the latest IA-2ic (unpublished) and GAD$_{65}$ antibody proficiency workshops, our assays had a sensitivity, specificity, validity, and consistency of 100% in each (21).

Undiluted sera were screened for ICA. The presence of ICA was established by testing serum with indirect immunofluorescence on a fresh group O cryofixed human pancreas (16). Positive tests were defined as ≥4 Juvenile Diabetes Foundation units. The ICA assay assessed in the ENDIT workshop (unpublished) had a sensitivity and specificity of 100%.

**HLA-DQB1 typing**

HLA-DQB1 was studied by a polymerase chain reaction restriction fragment–length polymorphism method combined with group-specific primers adapted from Nomura et al. (22). DNA was extracted from blood (23), and a 241-bp region from the polymorphic second exon of the DQB1 gene was amplified in two separate PCRs, specific for DQw1 and DQw2,3,4 alleles. Seven DQw1 and six DQw2,3,4 alleles can be identified after restriction against panels of seven and five restriction enzymes, respectively (22,24). The digested and undigested products were visualized by ethidium bromide staining after electrophoresis in a 4.2% agarose gel.

**INS**

INS was studied at the 1,127 Pst I polymorphic restriction site in the 3' untranslated region of the insulin gene and at the −23 Hinfl polymorphic restriction site (3). The digested and undigested products were visualized by ethidium bromide staining after electrophoresis in a 4.2% agarose gel. By convention, the disease associated allele at both sites is denoted as “+.”

![Table 1](https://example.com/table1.png)

**Table 1**—HLA-DQB1 allele positivity in monozygotic twins with type 1 diabetes

<table>
<thead>
<tr>
<th>Allele</th>
<th>% Concordant (n = 40)</th>
<th>% Discordant (n = 37)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0201</td>
<td>82.5 (n = 33)</td>
<td>65 (n = 24)</td>
<td>0.08</td>
</tr>
<tr>
<td>0301</td>
<td>2.5 (n = 1)</td>
<td>5.4 (n = 2)</td>
<td>NS</td>
</tr>
<tr>
<td>0302</td>
<td>60 (n = 24)</td>
<td>40.5 (n = 15)</td>
<td>0.09</td>
</tr>
<tr>
<td>0402</td>
<td>—</td>
<td>5.4 (n = 2)</td>
<td>NS</td>
</tr>
<tr>
<td>0501</td>
<td>—</td>
<td>2.7 (n = 1)</td>
<td>NS</td>
</tr>
<tr>
<td>0502</td>
<td>13 (n = 5)</td>
<td>5.4 (n = 2)</td>
<td>NS</td>
</tr>
<tr>
<td>0601</td>
<td>—</td>
<td>2.7 (n = 1)</td>
<td>NS</td>
</tr>
<tr>
<td>0602/3</td>
<td>2.5 (n = 1)</td>
<td>5.4 (n = 2)</td>
<td>NS</td>
</tr>
<tr>
<td>0604</td>
<td>—</td>
<td>5.4 (n = 2)</td>
<td>NS</td>
</tr>
<tr>
<td>0201/0302</td>
<td>47.5 (n = 19)</td>
<td>27 (n = 10)</td>
<td>0.06</td>
</tr>
<tr>
<td>Negative for DQB1*201 and/or *0302</td>
<td>5 (n = 2)</td>
<td>22 (n = 8)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

NS, not significant.

![Table 2](https://example.com/table2.png)

**Table 2**—23 Hinfl insulin genotypes in monozygotic twins with type 1 diabetes

<table>
<thead>
<tr>
<th>INS Genotypes</th>
<th>+/+</th>
<th>+/− (plus −/−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordant twins (n = 40)</td>
<td>0.875 (n = 35)</td>
<td>0.125 (n = 5)</td>
</tr>
<tr>
<td>Discordant twins (n = 37)</td>
<td>0.595 (n = 22)</td>
<td>0.405 (n = 15)</td>
</tr>
<tr>
<td>British caucasoid, ICA-negative controls (n = 63)*</td>
<td>0.54 (n = 34)</td>
<td>0.46 (n = 29)</td>
</tr>
<tr>
<td>Type 1 diabetes (n = 116)*</td>
<td>0.72 (n = 83)</td>
<td>0.28 (n = 33)</td>
</tr>
</tbody>
</table>

Concordant twins versus discordant twins (P = 0.005); concordant twins versus controls (P = 0.0001); concordant twins versus type 1 diabetes (P = 0.054); discordant twins versus controls (P = 0.68); discordant twins versus type 1 diabetes (P = 0.022); type 1 diabetes versus controls (P = 0.022). *Results taken from ref. 31. Patients with type 1 diabetes were serially selected from the Barts Oxford study, which is a population-based study of type 1 diabetes in the Oxfordshire region. The controls were all ICA-negative and were aged-matched to the subjects with type 1 diabetes. The disease-associated allele is designated (+).
Results are presented in Table 2. The type 22%; DQBI*0602/3. In contrast, 2 of 40 (5%) DQB1*0302, DQB1*0201, the conferences were found between concordant and discordant twins, as observed in our previous study (14), indicated that 37 twin pairs in each group would be sufficient to show a difference at the 5% level with 80% power. The study was approved by the ethical committee of the Barts and the London National Health Service Trust. Written and informed consent was received from the twins or their parents, as appropriate.

RESULTS

HLA DQB1 gene

Results are presented in Table 1. No differences were found between concordant and discordant twins for positivity of DQB1*0302, DQB1*0201, the combination of DQB1*0302/0201, and DQB1*0602/3. In contrast, 2 of 40 (5%) concordant twins compared with 8 of 37 (22%; P = 0.04) discordant possessed neither DQB1*0201 nor DQB1*0302.

INS

Results are presented in Table 2. The type 1 diabetes–associated +/+ genotype was increased for the 5' Hph I (P = 0.005; Table 2) polymorphism. Similarly, the 3' Pst I INS polymorphism was increased in frequency in discordant twins (frequency of +/+ genotype was 87.5% in concordant twins and 65% in discordant twins; P = 0.035).

HLA DQB1 and INS combined

Results are presented in Table 3. A total of 2 of 40 (5%) discordant twins compared with 12 of 37 (32.4%) discordant twins possessed neither DQB1*0201/DQB1*0302 nor the high-risk Hph I INS +/+ genotype (P = 0.002).

CONCLUSIONS — Our hypothesis was that discordance, compared with concordance, for type 1 diabetes in monozygotic twins is caused by a decreased load of both MHC and non-MHC susceptibility genes. From our observations, we conclude that the “load” of both MHC and non-MHC susceptibility genes does have an impact on disease penetrance of type 1 diabetes. Thus, the 5’ Hph I locus of the insulin gene was more prevalent in concordant than discordant twin pairs. Furthermore, there was an increased frequency of no high-risk HLA-DQB1 alleles in discordant compared with concordant twins. HLA-DQ protective alleles were as prevalent in concordant pairs as in discordant pairs. Lastly, only 5% discordant vs. 32.4% discordant twin pairs with type 1 diabetes possessed neither the HLA-DQB1*0302/0201 combination nor the INS Hph I high-risk genotype.

Differences between monozygous twins cannot be determined by germ-line genes. However, there are a number of ways genes could contribute to such disease discordance, including an increase in either protection or susceptibility to the disease. In the absence of a comprehensive understanding of the genes that contribute to genetic susceptibility to diabetes, the analysis of monozygotic twins discordant and concordant for diabetes provides a powerful method to assess the impact of a particular gene on disease expression.

Using DQB1 typing, we have confirmed the importance of the total load of MHC alleles as a determinant of concordance of disease, although we have been unable to confirm our previous results by studying individual DQB1 alleles compared with the previous DR serological typing (14). In that previous study, we found an increased frequency of the het-

Table 3—Hph I high-risk insulin genotype and HLA DQB1*0201/*0302 combinations in monozygotic twins with type 1 diabetes

<table>
<thead>
<tr>
<th>Twins</th>
<th>INS negative</th>
<th>INS positive</th>
<th>INS negative</th>
<th>INS positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DQ negative*</td>
<td>DQ positive</td>
<td>DQ negative</td>
<td>DQ positive</td>
</tr>
<tr>
<td>Concordant (n = 40)</td>
<td>0.05 (n = 2)</td>
<td>0.40 (n = 16)</td>
<td>0.075 (n = 3)</td>
<td>0.475 (n = 19)</td>
</tr>
<tr>
<td>Discordant (n = 37)</td>
<td>0.324 (n = 12)</td>
<td>0.189 (n = 7)</td>
<td>0.081 (n = 3)</td>
<td>0.405 (n = 15)</td>
</tr>
</tbody>
</table>

INS, possession of Hph I high-risk genotype (+/+); DQ, possession of HLA DQB1*0201 and/or *0302. Overall Pearson $\chi^2$, $P = 0.012$, likelihood exact $\chi^2$, $P = 0.011$. $\ast2 \times 2$ Pearson $\chi^2$, $P = 0.002$.

Statistics

All statistical analyses were performed using SPSS for Windows software (version 9; Chicago, IL). Differences in frequencies of genotypes and alleles were calculated by $\chi^2$ analysis; all $P$ values quoted are two-sided. Power analysis based on a 31% difference of DR3/DR4 heterozygotes between concordant and discordant twins, as seen in our previous study (14), indicated that 37 twin pairs in each group would be sufficient to show a difference at the 5% level with 80% power. The study was approved by the ethical committee of the Barts and the London National Health Service Trust. Written and informed consent was received from the twins or their parents, as appropriate.

HLA DQBI gene

Results are presented in Table 1. No differences were found between concordant and discordant twins for positivity of DQB1*0302, DQB1*0201, the combination of DQB1*0302/0201, and DQB1*0602/3. In contrast, 2 of 40 (5%) concordant twins compared with 8 of 37 (22%; $P = 0.04$) discordant possessed neither DQB1*0201 nor DQB1*0302.

INS

Results are presented in Table 2. The type 1 diabetes–associated +/+ genotype was increased for the 5’ Hph I ($P = 0.005$; Table 2) polymorphism. Similarly, the 3’ Pst I INS polymorphism was increased in frequency in discordant twins (frequency of +/+ genotype was 87.5% in concordant twins and 65% in discordant twins; $P = 0.035$).

HLA DQB1 and INS combined

Results are presented in Table 3. A total of 2 of 40 (5%) discordant twins compared with 12 of 37 (32.4%) discordant twins possessed neither DQB1*0201/DQB1*0302 nor the high-risk Hph I INS +/+ genotype ($P = 0.002$).

CONCLUSIONS — Our hypothesis was that discordance, compared with concordance, for type 1 diabetes in monozygotic twins is caused by a decreased load of both MHC and non-MHC susceptibility genes. From our observations, we conclude that the “load” of both MHC and non-MHC susceptibility genes does have an impact on disease penetrance of type 1 diabetes. Thus, the 5’ Hph I locus of the insulin gene was more prevalent in concordant than discordant twin pairs. Furthermore, there was an increased frequency of no high-risk HLA-DQB1 alleles in discordant compared with concordant twins. HLA-DQ protective alleles were as prevalent in concordant pairs as in discordant pairs. Lastly, only 5% discordant vs. 32.4% discordant twin pairs with type 1 diabetes possessed neither the HLA-DQB1*0302/0201 combination nor the INS Hph I high-risk genotype.

Differences between monozygous twins cannot be determined by germ-line genes. However, there are a number of ways genes could contribute to such disease discordance, including an increase in either protection or susceptibility to the disease. In the absence of a comprehensive understanding of the genes that contribute to genetic susceptibility to diabetes, the analysis of monozygotic twins discordant and concordant for diabetes provides a powerful method to assess the impact of a particular gene on disease expression.

Using DQB1 typing, we have confirmed the importance of the total load of MHC alleles as a determinant of concordance of disease, although we have been unable to confirm our previous results by studying individual DQB1 alleles compared with the previous DR serological typing (14). In that previous study, we found an increased frequency of the het-
transcription could depend on sequence variation in the disease-associated variable region, although different studies have revealed discrepant results, depending on the reporter construct and the in vivo/vitro models used (25–30). Interestingly, whereas twins discordant for diabetes had a higher frequency of disease-associated insulin genotypes than discordant pairs, twins discordant for diabetes did not have a greater frequency than did the islet cell–negative control population (31) (Table 2). This supports the hypothesis that there is a decreased load of diabetes susceptibility genes in discordant compared with concordant twins. Last, this study would also support the interaction of HLA-DQ and insulin gene alleles in determining susceptibility and protection to type 1 diabetes.

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References


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