HLA-DQB1–defined Genetic Susceptibility, Beta Cell Autoimmunity, and Metabolic Characteristics in Familial and Nonfamilial Insulin-dependent Diabetes Mellitus

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Abstract

Familial aggregation of insulin-dependent diabetes mellitus (IDDM) is a common phenomenon, but the reasons behind it are poorly understood. To investigate whether there is heterogeneity between familial and nonfamilial forms of IDDM we compared genetic, immunological, and clinical characteristics of diabetic children with and without an affected first-degree relative in a population-based series of Finnish children with IDDM. The frequencies of HLA-DQB1 genotypes known to be associated with high (DQB1-*0302/0201) or moderate (*0302/x) IDDM risk in the Finnish population were increased, while the proportions of DQB1 genotypes associated with low or decreased risk for IDDM were reduced in the 121 familial cases as compared with the 574 nonfamilial cases (32.7 vs. 21.3%, 41.3 vs. 35.9%, 18.3 vs. 31.4%, and 7.7 vs. 11.4%, respectively; \( P = 0.002 \)). The frequencies and serum concentrations of islet cell antibodies, insulin autoantibodies, and antibodies to the 65-kD isoform of glutamic acid decarboxylase were similar at diagnosis in the familial and nonfamilial cases. The 31 first-affected cases in the multiple case families were younger at diagnosis than the nonfamilial cases (6.9 vs. 8.5 yr; \( P < 0.05 \)). The 90 second-affected familial cases had less severe metabolic decompensation at diagnosis than either the first-affected familial or nonfamilial cases. In conclusion, familial aggregation of IDDM in Finland is at least partly explained by a higher frequency of IDDM susceptibility genes in families with multiple affected individuals. The lack of differences in autoantibody levels between the familial and nonfamilial cases indicates homogeneity rather than heterogeneity in the pathogenetic process of beta cell destruction. (J. Clin. Invest. 1996. 98:2489–2495.) Key words: genetic heterogeneity • autoantibodies • diabetic ketoacidosis • C-peptide • glycated hemoglobin A

Introduction

Familial aggregation of insulin-dependent diabetes mellitus (IDDM) is a well-established phenomenon demonstrated in many epidemiological studies. The proportion of subjects with IDDM having one or more affected first-degree relatives has fluctuated considerably, however (1–5), probably due to small sample sizes, selection bias, short follow-up periods, variations in family size, and other methodological problems. More recently, population-based data have become available, and fairly consistent prevalence rates of 11–13% for the presence of an affected first-degree relative have been reported in subjects with IDDM at the time of diagnosis (6–8). The risk of IDDM in siblings of diabetic probands up to the age of 30 yr has been estimated to be \( \sim 6–7\% \) (9–12), although a higher long-term risk, 9.6% up to the age of 60 yr, has been reported recently in Denmark (12). Approximately 4% of the parents of IDDM patients (6, 7, 10) and 5% of the offspring are affected by the disease (1, 13). Intriguingly, it has been observed that it is more often the father than the mother of a child with IDDM who is affected (7, 8, 14).

The reasons for these epidemiological observations are unknown, but it has been suggested that familial cases are genetically more susceptible to IDDM, and that as yet unidentified environmental risk factors have clustered in certain families with multiple affected individuals. However, there have been several studies that have failed to detect differences in the frequency of the high risk HLA phenotypes between IDDM cases with and without an affected family member (2, 3, 15–17).

Because of the above-mentioned methodological problems and varying definitions used for both familial and sporadic IDDM cases, most sets of previous results are difficult to compare. Clearly, population-based studies are needed to find out whether there is heterogeneity between familial and nonfamilial forms of IDDM.

Methods

Patient population. The population-based, nationwide “Childhood Diabetes in Finland” (DiMe) Study was started in 1986 to investigate the role of genetic, immunological, and environmental factors in the development of IDDM. From the beginning of September 1986 to the end of April 1989, 801 index cases aged 14 yr or younger were diagnosed with IDDM and invited to participate together with their fami—}
anti–human IgG (Behringwerke AG, Marburg, Germany) was used for samples that were positive for ICA and the results were expressed in Juvenile Diabetes Foundation units (JDF-U) relative to an international reference standard (19). The detection limit for ICA was 2.5 JDF-U. Our laboratory has participated in the international workshops on the standardization of ICA methods, in which its sensitivity was 100%, specificity 98%, validity 98%, and consistency 98% in the fourth round.

Antibodies to the 65-kD isof orm of glutamic acid decarboxylase (GADA) were measured using a competitive radioligand assay recently described by Petersen et al. (20). The limit for GADA positivity was a specific binding of 6.5 relative units (RU), representing the 99th percentile in a series of 372 healthy control children. The disease sensitivity of the present assay was 80% and the specificity 94% based on the 101 samples included in the second international GAD antibody workshop (21).

HLA-DQB1 typing was performed by a previously described method based on time-resolved fluorescence (22). Four sequence-specific oligonucleotide probes were used to identify the following DQB1 alleles known to be significantly associated with either susceptibility to or protection against IDDM in the Finnish population: DQB1*0302, DQB1*0201, DQB1*0602 or 0603, and DQB1*0301 (23). The recently described simplified classification of DQB1 genotypes into high risk (DQB1*0302/0301), moderate risk (DQB1*0302/x; x means 0302 or a nondefined allele), low risk (DQB1*0301/0302, DQB1*0101/0301, DQB1*0201/x, DQB1*0302/0602-3; x means 0201 or a nondefined allele), and decreased risk (DQB1*xx, DQB1*0301/x, DQB1*0201/0602-3, DQB1*0301/0602-3, DQB1*0602-3/x; x means a nondefined allele) genotypes was used (23). HLA-DQB1 typing data were available for 661 index cases (82.5%).

Results

The median follow-up time for the families up to the end of October 1995 was 7.7 yr (range 6.5–9.2 yr). The number of biological siblings had increased by 104 in this time, representing children born into the families after the diagnosis of the index case. Altogether 45 additional first-degree relatives, comprising 43 siblings and two fathers in 40 families, had developed IDDM during the study. Thus the total number of families with multiple IDDM cases was 121 (15.1%) at the end of the follow-up, the frequencies of affected fathers and mothers being 5.9 and 2.6%, respectively, and the proportion of the siblings affected 6.1% (71/1,168).

About half of these 121 familial cases were multiplex cases (n = 62) and the others were multigenerational cases (n = 59). Nine probands having both a parent and a sibling with IDDM were included among the multiplex cases. The familial cases included 31 first-affected probands and 90 second-affected ones. After exclusion of the index cases with no biological siblings, a series of 574 nonfamilial cases remained. The classification of the index cases has been described in Fig. 1.

The basic epidemiological characteristics, distribution of genetic markers, occurrence of IDDM-associated autoantibodies, and degree of metabolic compensation at diagnosis were compared between the 121 familial and 574 nonfamilial IDDM cases. No difference was observed in the seasonal inci-
dence of IDDM between these groups, both showing the lowest incidence in summer. Neither were any significant differences observed in the sex distribution or the mean age at diagnosis between the familial and nonfamilial cases. Subdivision of the 121 familial subjects into 62 multiplex and 59 multigenerational cases revealed no significant differences in age at diagnosis (Table I), whereas the 31 first-affected familial cases were found to be significantly younger at diagnosis than the nonfamilial cases (Table II).

The distribution of high, moderate, low, and decreased risk HLA-DQB1 genotypes differed significantly between the familial and nonfamilial cases ($P = 0.002$, Fig. 2). Considering single DQB1 genotypes the frequency of the DQB1*0302/0201 genotype was increased among the familial cases (32.7 vs. 21.3% in nonfamilial cases; $P = 0.002$). No differences were observed in the frequencies of other DQB1 genotypes (Table II). The 31 first-affected familial cases had the same degree of metabolic decomposition as the nonfamilial cases ($P = 0.06$ and 0.007; respectively), but no difference was observed between the first and second-affected cases (data not shown). There was no difference in the distribution of the four DQB1 defined risk genotypes between various age groups at the diagnosis of IDDM (Fig. 3).

No differences in either the prevalence or serum concentrations of ICA, IAA, or GADA at diagnosis were observed between the familial and nonfamilial cases. The frequencies and circulating levels of these autoantibodies in the nonfamilial, multiplex, and multigenerational IDDM cases are shown in Table I.

The 121 familial cases had lower blood glucose and GHb levels at diagnosis and a reduced frequency of ketoacidosis as compared with the nonfamilial cases [17.8 mmol/liter [inter-quartile range (IQR) 12.4–22.9 mmol/liter] vs. 20.8 mmol/liter [IQR 15.8–26.9 mmol/liter]; $P < 0.001$, 10.5 SDS [95% confidence interval (CI) 9.3–11.6]; vs. 13.2 SDS [95% CI 12.6–13.8]; $P < 0.001$, and 9.1% [95% CI 3.7–14.5] vs. 25.5% [95% CI 21.8–29.2]; $P < 0.001$, respectively]. Higher serum C-peptide concentrations at diagnosis and lower insulin doses during the initial 24 h were also observed among the familial cases [0.19 nmol/liter (IQR 0.17–0.30) vs. 0.15 nmol/liter (IQR 0.10–0.24); $P = 0.008$ and 0.93 IU/kg [95% CI 0.81–1.07] vs. 1.25 IU/kg [95% CI 1.19–1.31]; $P < 0.001$]. Subdivision of the familial cases into first- and second-affected ones showed that the 90 second-affected familial cases had lower blood glucose and GHb levels, a lower frequency of ketoacidosis, higher serum concentrations of C-peptide, and lower insulin dosage during the initial 24 h than the nonfamilial cases, whereas the 31 first-affected familial cases had the same degree of metabolic dec complication as the nonfamilial cases (Table II). No differences were observed between these groups in either serum

### Table I. Clinical, Genetic, and Immunological Characteristics of Familial and Nonfamilial IDDM Cases at Diagnosis

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Multiplex $n = 62$</th>
<th>Multigenerational $n = 59$</th>
<th>Nonfamilial cases $n = 574$</th>
<th>Cases with no biological siblings $n = 106$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>34/28</td>
<td>26/59</td>
<td>327/247</td>
<td>53/53</td>
</tr>
<tr>
<td>Age, yr (95% CI)</td>
<td>7.7 (6.8–8.6)</td>
<td>8.3 (7.3–9.3)</td>
<td>8.5 (8.2–8.8)</td>
<td>8.4 (7.9–7.2)</td>
</tr>
<tr>
<td>HLA-DQB1 genotypes$^a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High risk, $n$ (%)</td>
<td>22 (37.9)</td>
<td>12 (26.1)</td>
<td>101 (21.3)</td>
<td>25 (30.1)</td>
</tr>
<tr>
<td>Moderate risk, $n$ (%)</td>
<td>21 (36.2)</td>
<td>22 (47.8)</td>
<td>170 (35.9)</td>
<td>32 (38.6)</td>
</tr>
<tr>
<td>Low risk, $n$ (%)</td>
<td>11 (19.0)</td>
<td>8 (17.4)</td>
<td>149 (31.4)</td>
<td>19 (22.9)</td>
</tr>
<tr>
<td>Decreased risk, $n$ (%)</td>
<td>4 (6.9)</td>
<td>4 (8.7)</td>
<td>54 (11.4)</td>
<td>7 (8.4)</td>
</tr>
</tbody>
</table>

Serum autoantibodies

<table>
<thead>
<tr>
<th></th>
<th>Multiplex $n = 62$</th>
<th>Multigenerational $n = 59$</th>
<th>Nonfamilial cases $n = 574$</th>
<th>Cases with no biological siblings $n = 106$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA, % (95% CI)</td>
<td>83.6 (74.3–92.9)</td>
<td>81.0 (70.9–91.1)</td>
<td>84.4 (81.4–87.4)</td>
<td>84.6 (77.7–91.5)</td>
</tr>
<tr>
<td>ICA, JDF-U (IQR)</td>
<td>34 (9–160)</td>
<td>36 (9–88)</td>
<td>36 (10–141)</td>
<td>36 (13–129)</td>
</tr>
<tr>
<td>IAA, % (95% CI)</td>
<td>50.8 (38.1–63.6)</td>
<td>43.9 (31.0–56.7)</td>
<td>49.8 (45.6–54.0)</td>
<td>44.6 (34.9–54.3)</td>
</tr>
<tr>
<td>IAA, nU/ml (IQR)</td>
<td>57 (30–156)</td>
<td>47 (24–137)</td>
<td>54 (28–146)</td>
<td>43 (14–125)</td>
</tr>
<tr>
<td>GADA, % (95% CI)</td>
<td>72.4 (60.9–83.9)</td>
<td>74.1 (62.8–85.4)</td>
<td>73.1 (69.4–76.8)</td>
<td>77.7 (69.7–85.7)</td>
</tr>
<tr>
<td>GADA, RU (IQR)</td>
<td>15.4 (4.9–32.5)</td>
<td>16.7 (6.4–45.4)</td>
<td>21.3 (5.8–65.2)</td>
<td>32.4 (8.0–90.9)</td>
</tr>
</tbody>
</table>

Multiplex cases have a sibling with IDDM, multigenerational cases have an affected parent, and nonfamilial cases are subjects with at least one sibling but no first-degree relatives with IDDM. A simplified classification into high risk (DQB1*0302/0201), moderate risk (DQB1*0302/x; x means 0302 or a nondefined allele), low risk (DQB1*0301/0302, 0201/0301, 0201/x, 0302/0602-3; x means 0201 or a nondefined allele), and decreased risk (DQB1*x/x, 0301/x, 0201/0602-3, 0301/0602-3, 0602-3/x; x means a nondefined allele) DQB1 genotypes was used. Values are frequencies (95% CI), means (95% CI), or medians (IQR). $^a P = 0.004$ for comparison of DQB1 genotype distribution between multiplex and nonfamilial cases; $^b P = 0.09$ for comparison of DQB1 genotype distribution between multigenerational and nonfamilial cases; $^c$DQB1 typing data available in 58 multiplex cases, 46 multigenerational cases, 474 nonfamilial cases, and 83 cases with no biological siblings.
C-peptide concentrations or daily insulin doses during the initial 2 yr of IDDM, whereas GHb levels were significantly higher during the second year among the second-affected familial cases than in either of the other groups (Table II).

All the analyses were repeated after including the 106 index cases with no biological siblings in the group of nonfamilial cases, but this did not change the above-mentioned results. Neither were any differences observed in the results when the nine cases having both a parent and a sibling with IDDM were considered as multigenerational cases.

**Discussion**

Opportunities to study the etiology of human IDDM are optimal in Finland because the incidence of IDDM is the highest in the world (24), the population has a culturally and genetically homogenous background, and health care for patients with diabetes is well organized. In the nationwide DiMe study the proportion of children with IDDM having an affected first-degree relative at the time of diagnosis was 11.2% (7), but after a median follow-up period of 7.7 yr, 15.1% of the index cases had a first-degree relative with the disease. These figures are in good accordance with the data of a recent Danish study reporting that 22.4% of subjects with childhood-onset IDDM...
and disease duration > 30 yr have a first-degree relative with IDDM (12).

The present investigation was aimed at searching for evidence of possible heterogeneity between familial and nonfamilial IDDM cases. A review of previous studies focusing on this topic reveals that varying definitions for both familial and nonfamilial forms of IDDM have been used, and therefore interpretation and comparison of these studies is complicated. We defined a nonfamilial or sporadic IDDM case as a diabetic proband with at least one biological sibling but no first-degree relatives having IDDM. Thus families with a single child with IDDM and both parents unaffected were excluded from the analysis, since they can be considered as potentially multiplex families.

The familial cases were slightly younger at diagnosis of IDDM, the difference being statistically significant when the 31 first-affected familial cases were compared with the nonfamilial cases (6.9 vs. 8.5 yr). These results are in line with those of a recent Danish study (8), which also reported a lower mean age at diagnosis in the first-affected children in multiple case families than in sporadic cases (7.6 vs. 8.6 yr). In that study the second-affected siblings were observed to be significantly older at diagnosis (11.2 yr) than the first-affected familial cases. Two reports from Pittsburgh quote an older age at diagnosis among second-affected cases than among nonfamilial cases. Two reports from Pittsburgh quote an older age at diagnosis among second-affected cases than among nonfamilial cases. Two reports from Pittsburgh quote an older age at diagnosis among second-affected cases than among nonfamilial cases. Two reports from Pittsburgh quote an older age at diagnosis among second-affected cases than among nonfamilial cases.

### Table III. The Frequencies of Various DQB1 Genotypes and DQB1 Alleles in Familial and Nonfamilial IDDM Cases

<table>
<thead>
<tr>
<th>DQB1 genotype or allele</th>
<th>Familial cases n (%)</th>
<th>Nonfamilial cases n (%)</th>
<th>$\chi^2$ statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>0302/0201</td>
<td>34 (32.7)</td>
<td>101 (21.3)</td>
<td>$P = 0.02$</td>
</tr>
<tr>
<td>0302/x</td>
<td>43 (41.3)</td>
<td>170 (35.9)</td>
<td>NS</td>
</tr>
<tr>
<td>0301/0302</td>
<td>3 (2.9)</td>
<td>32 (6.8)</td>
<td>NS</td>
</tr>
<tr>
<td>0201/0301</td>
<td>3 (2.9)</td>
<td>26 (5.5)</td>
<td>NS</td>
</tr>
<tr>
<td>0201/x</td>
<td>11 (10.6)</td>
<td>66 (13.9)</td>
<td>NS</td>
</tr>
<tr>
<td>0302/0602-3</td>
<td>2 (1.9)</td>
<td>25 (5.3)</td>
<td>NS</td>
</tr>
<tr>
<td>x/x</td>
<td>3 (2.9)</td>
<td>29 (6.1)</td>
<td>NS</td>
</tr>
<tr>
<td>0301/x</td>
<td>—</td>
<td>15 (3.2)</td>
<td>NS</td>
</tr>
<tr>
<td>0201/0602-3</td>
<td>2 (1.9)</td>
<td>4 (0.8)</td>
<td>NS</td>
</tr>
<tr>
<td>0301/0602-3</td>
<td>3 (2.9)</td>
<td>2 (0.4)</td>
<td>NS</td>
</tr>
<tr>
<td>0602-3/x</td>
<td>—</td>
<td>4 (0.8)</td>
<td>NS</td>
</tr>
<tr>
<td>0302</td>
<td>82 (78.8)</td>
<td>328 (69.2)</td>
<td>$P = 0.065$</td>
</tr>
<tr>
<td>0201</td>
<td>50 (48.1)</td>
<td>197 (41.6)</td>
<td>NS</td>
</tr>
<tr>
<td>0301</td>
<td>9 (8.7)</td>
<td>75 (15.8)</td>
<td>$P = 0.09$</td>
</tr>
<tr>
<td>0602-3</td>
<td>7 (6.7)</td>
<td>35 (7.4)</td>
<td>NS</td>
</tr>
</tbody>
</table>

DQB1 typing data were available for 104 familial cases and 474 nonfamilial cases.
IDDM as compared with nonfamilial cases. Earlier, only Svejgaard et al. (29) had been able to show a significantly elevated frequency of the DR3/4 phenotype among familial cases, but the difference in the frequency of the DQB1*0302/0201 genotype between familial and sporadic IDDM cases in Denmark was not significant in a recent nationwide survey (42 vs. 34%, respectively) (16). However, the present series is the first population-based study in which the frequencies of various DQB1 genotypes have been compared between familial and nonfamilial cases, and our study population is also considerably larger than the Danish one. It may also be possible that there are differences between populations in genetic and/or environmental factors which explain the clustering of IDDM in families.

Previous studies have indicated that there are differences in the frequencies of high risk HLA class II genotypes between childhood and adult-onset IDDM cases, the proportion of DR3/DR4 heterozygotes being higher in subjects with young age at diagnosis (4, 30). Our study population included only childhood-onset cases, and when analyzing the distribution of the four DQB1-defined risk genotypes in different age groups no significant difference could be observed. Accordingly, the age at diagnosis can hardly induce any bias when comparing the frequencies of various DQB1 genotypes between familial and nonfamilial cases.

In this study no differences were found in the overall distribution of DQB1 genotypes between the multiplex and multigenerational or first-affected and second-affected familial cases, but the highest frequency of the DQB1*0302/0201 genotype was observed among the multiplex cases, while the DQB1*0302/x genotype was the most common one in the multigenerational cases (Table I). This observation could indicate that the affected offspring in the multigenerational cases have inherited the DQB1*0302-positive haplotype from the affected parent, while in multiplex cases it would be the DQB1*0302/0201 combination which is primarily responsible for the enhanced IDDM risk.

Although our results show that affected subjects in multiple case families are genetically more susceptible to IDDM, the role of environmental factors in the familial aggregation of IDDM cannot be ruled out. In fact, it is likely that as yet unidentified genetic–environmental interactions are the most important elements explaining the development of IDDM in subjects from the same families. Some evidence has already been obtained for a possible link between certain enteroviruses and high risk HLA genotypes, by virtue of the observation that the frequency of antibodies to Coxsackie-B viruses was higher in newly diagnosed IDDM subjects with the HLA-DR4 than in those with other DR types (31, 32).

Various autoantibodies, like ICA, IAA, and GADA, have been used as immunological markers of the development of IDDM when aiming for prediction and prevention of IDDM. The lack of a difference in either the occurrence of these autoantibodies or their serum levels between familial and sporadic cases suggests that similar pathogenetic processes resulting in the appearance of various autoantibodies take place in the prediabetic period in subjects with or without a family history of IDDM. Accordingly, observations on the evolution of IDDM obtained mainly from first-degree relatives of subjects with the disease are likely to be applicable to sporadic cases, although there are differences in the predictive value of various IDDM-associated autoantibodies between families and the general population (33, 34).

The absence of any differences in the degree of metabolic decompensation at diagnosis between the first-affected familial and nonfamilial cases, whereas the second-affected familial cases had milder metabolic alterations, indicates that the favorable metabolic condition among those with a family history of IDDM is due to the increased awareness of the disease and its symptoms in the family and confirms the results reported previously by O’Leary et al. (17). The higher GHb levels in the second-affected familial cases observed during the second year of IDDM may be related to psychological factors reflected by a higher acceptance rate of hyperglycemia and fear of hypoglycemia as a consequence of previous experiences.

In conclusion, the increased frequency of DQB1*0302/0201 genotype found in familial IDDM cases partly explains the familial aggregation of the disease. No differences were observed either in the frequencies or serum levels of IDDM-associated autoantibodies between familial and nonfamilial cases, and the degree of metabolic decompensation was similar between the first-affected familial and nonfamilial cases. Consequently, a homogeneous rather than a heterogeneous pathogenetic process seems to be responsible for beta cell destruction in familial and nonfamilial IDDM.

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References


