HLA-DQ Genotypes in Classic Type 1 Diabetes and in Latent Autoimmune Diabetes of the Adult

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In 1993–1996, islet autoantibodies, C-peptide, and HLA-DQ genotypes were evaluated in 345 insulin-treated diabetic patients of all ages from the Skaraborg Diabetes Registry 5–6 years after their diagnosis and in 216 control subjects from the Skaraborg County, Sweden, population. The aims of this study were to clarify the importance of age at diagnosis of diabetes for HLA-DQ associations in patients with classic type 1 diabetes and whether patients considered to have latent autoimmune diabetes of the adult differed in their human leukocyte antigen (HLA) associations. An abnormally low fasting C-peptide value was used as the definition of type 1 diabetes, found in 182 of 345 (53%) patients. No major associations between age at diagnosis and HLA susceptibility or protective genotypes were detected in type 1 diabetic patients. Among the 163 patients with preserved β-cell function, the frequency of HLA protective genotypes was clearly decreased (5% vs. 42%) in the 46 of 163 with islet antibodies compared with the 117 of 163 antibody-negative patients. The authors conclude that there were no major effects of age at diagnosis on HLA-DQ associations in classic type 1 diabetic patients, whereas lack of HLA-DQ protective genotypes was a feature of patients with slow-progressing type 1 diabetes (latent autoimmune diabetes of the adult).

currently considered type 1 diabetes. Later, using refined techniques, others (2–7) described both positive and nega-
tive HLA associations with type 1 diabetes. Previous HLA studies in type 1 diabetic patients have been focused on children (8–15). Type 1 diabetes, however, affects individuals of all ages. Indeed, 44 percent of patients developing type 1 diabetes are above 30 years of age at the time of diagnosis (16). Despite this, HLA studies in adult type 1 diabetic patients are rare. In fact, besides the study by Caillat-Zucman et al. (17), only a small series of patients diagnosed as adults have been reported (18–22). Some of these studies infer that the high-risk genotype DQA1-DQB1 *0501-*0201/*0301-*0302 is less frequent in adult type 1 diabetic patients than in children (9–11, 14, 15, 19, 22) and that the frequency of protective genotypes increases with the age at onset of type 1 diabetes (15).

Studies of type 1 diabetes in adult patients are complicated by the fact that type 2 diabetes is the most frequent form of diabetes in this age group. Recent studies have shown that, without an assessment of islet antibodies, it is difficult to distinguish type 1 from type 2 diabetes (23–26). Patients thought to have type 2 diabetes (phenotypic type 2 diabetes) in combination with islet antibodies are prone to develop β-cell failure (23, 27, 28). This type of diabetes has been labeled slow-progressing type 1 diabetes (29), type 1½ diabetes (30), latent autoimmune diabetes in adults (LADA) (31, 32), or autoimmune diabetes not requiring insulin at diagnosis (33). The question of whether these patients have type 1 diabetes, type 2 diabetes, or type 2 diabetes complicated by type 1 diabetes, as recently suggested (30, 34), has to be answered. HLA genotyping in this context may be rewarding. Indeed, it was recently reported that the frequency of HLA genotypes negatively associated with type 1 diabetes (protective HLA types) was increased in LADA patients (15, 26).

To examine the effect of age at diagnosis on HLA associations in classic type 1 diabetes, we evaluated β-cell function in a population-based group of type 1 diabetic patients 5–6 years after diagnosis when β-cell failure should be overt (28). In addition, islet antibodies were measured to test the hypothesis that antibody-positive diabetic patients with preserved β-cell function 5–6 years after diagnosis of diabetes may have different HLA associations than do autoimmune type 1 diabetic patients with obvious β-cell failure (i.e., with classic type 1 diabetes). The aims of this study were to clarify whether the relation between HLA genotypes and type 1 diabetes is affected by age at diagnosis and, in patients with preserved β-cell function, by the presence of islet antibodies.

MATERIALS AND METHODS

Patients

The Skaraborg Diabetes Registry (established in 1991), serving the county of Skaraborg (280,000 inhabitants), Sweden, is a reliable diabetes registry (96 percent ascertainment) (35). As of December 31, 1995, there were 9,941 patients registered in the Skaraborg Diabetes Registry; 3,362 new cases were diagnosed during the 7-year period from January 1, 1985, to December 31, 1991. Among the 3,362 new cases, 557 (17 percent) were started on continuous insulin treatment immediately or later after the diagnosis of diabetes (mean, 0.9 years, standard deviation (SD), 1.6 years; range, 0–6.7 years). The current follow-up study was conducted in 1993–1996 when the mean duration of diabetes was 5.6 (SD, 2.1) years (range, 1.3–10.7 years) in the patients. After diagnosis and before follow-up, 52 of 557 insulin-treated patients had died, and 26 of 557 had moved out of the county. Accordingly, 479 patients from the original cohort (86 percent) were invited to the follow-up study, and 356 of them accepted and completed the investigation. It was, however, discovered at the follow-up that 10 of 356 participants had secondary diabetes and one had missing data. Therefore, altogether 345 of 479 eligible patients (72 percent) participated in this study; 161 were women (47 percent) and 82 were children (36 girls; 44 percent; below 15 years of age) when diagnosed as a diabetic patient. Using the World Health Organization definition (36) at the time of registration in the Skaraborg Diabetes Registry, the reporting physicians considered 191 of the included patients to have type 1 and 154 to have type 2 diabetes.

Control subjects

Nondiabetic controls for the patients younger than 15 years at diagnosis were selected according to the World Health Organization DIAMOND Molecular Epidemiology Project protocol (37). Following this protocol, 101 individuals treated with minor surgery unlikely to have any association with the HLA system acted as controls (matched for age and gender) to the 82 diabetic children. Nondiabetic controls for patients diagnosed above 15 years of age were obtained from the Skaraborg Population Registry; a 1 percent sample of the population aged 15 years or more (2,300 individuals) was available as potential controls. From this population, two subjects matched for age and gender to each diabetic proband (n = 246) were invited, and 118 agreed to participate in the investigation. Three of the selected control subjects had diabetes and had to be excluded. Hence, the nondiabetic control population comprised 216 subjects (age: mean, 29.8 (SD, 13.5) years; range, 15.7–81.2 years; 103 (47.7 percent) females). The study was approved by the Ethics Committee, Göteborg University. All participants gave their informed consent prior to their inclusion in the study.

Methods

Omitting the morning insulin injection before vein puncture, we obtained a blood sample from each of the 345 participating patients after an overnight fast. This was immediately followed by an intravenous injection of 1 mg of glucagon in 254 of 345 (73.6 percent) patients, and a postglucagon blood sample was taken 6 minutes later. The main reason for not doing glucagon testing in the remaining 91 patients was to avoid glucagon side effects in young or elderly patients: 84 of 91 (92.3 percent) patients not tested with glucagon were below 20 years of age or above 65 years. A blood sample was obtained from 216 controls, with the sample being taken from 114 of these while fasting. Among the controls, HLA genotyping was conducted in 216, autoan-
Glutamic acid decarboxylase antibody

Islet cell antibodies

C-peptide

Serum C-peptide was measured by routine methods: RIA-gnost C-peptide (Hoechst AG, Marburg, Germany), with a detection limit of 0.10 nmol/liter (coefficient of variation, 8.8 percent at the 0.48-nmol/liter level) during the first part of the study, and C-PEP-CT2 (CIS Bio International, Marcoule, France), with a detection limit of 0.025 nmol/liter (coefficient of variation, 8.9 percent at the 0.64-nmol/liter level), from January 1, 1995. In the 114 control subjects with a fasting blood sample, the mean fasting serum C-peptide level was 0.67 (SD, 0.26) nmol/liter; the range, 0.3–1.9 nmol/liter. Values lower than the mean of –1.64 nmol/liter (one-sided test) for the controls were considered abnormally low (≤0.24 nmol/liter). A postglucagon serum C-peptide level of less than or equal to 0.6 nmol/liter was considered abnormal (≤0.6 nmol/liter). Statistical analyses

Comparisons between group frequencies were performed by use of the chi-square test or Fisher’s exact test with corrections of p values for multiple comparisons when appropriate. Group comparisons with respect to age, diabetes duration, serum C-peptide, insulin dose, body mass index, and so on were performed using analysis of variances (fractional and post hoc Sheffe’s test). Correlation between fasting and stimulated C-peptide was tested by Spearman’s rank correlation. Data are reported as the mean and standard deviation. Logarithmic age was used for univariate analysis of variance between age associations with type 1 diabetes-promoting and -protective genotypes. Odds ratios and 95 percent confidence intervals were calculated for the different genotypes versus controls with genotype X/X as baseline (odds ratio (OR) = 1.00) by use of the formula ad/bc, where a is the number of patients with one of the genotypes DQA1*0301-DQB1*0302/DQA1*0501-DQB1*0201, DQA1*0301-DQB1*0302/X, *0501-DQB1*0201/X, or DQA1*0102(3)-DQB1*0602(3)/X; b is the number of control subjects with the corresponding genotype; c is the number of diabetic patients with the nondiabetogenic genotype X/X; and d is the number of control subjects with the nondiabetogenic genotype X/X.

RESULTS

Antibodies

Islet cell antibodies were detected in 88 patients, 67 clinically considered as having type 1 and 21 as having type 2 diabetes, respectively, whereas glutamic acid decarboxylase antibodies were detected in 141 patients, 100 considered as having clinical type 1 and 41 as having clinical type 2...
diabetes. Islet cell antibodies and glutamic acid decarboxylase antibodies were detected simultaneously in 70 patients (50 with clinical type 1, 20 with clinical type 2), and islet cell antibodies or glutamic acid decarboxylase antibodies were detected in 85 diabetic patients (65 with clinical type 1, 20 with clinical type 2). Consequently, islet cell antibodies and/or glutamic acid decarboxylase antibodies were present in 157 diabetic patients (116 with clinical type 1, 41 with clinical type 2).

C-peptide and clinical characteristics

The 345 diabetic patients could be divided into two groups according to their fasting C-peptide values: diabetic patients with low C-peptide values (≤0.24 nmol/liter; CPEP-LOW) and those with normal C-peptide values (>0.24 nmol/liter; CPEP-NORM). CPEP-LOW comprised 182 patients (111/182 patients (61 percent) with islet antibodies (CPEP-LOW Ab+) and 71/182 patients (39 percent) without (CPEP-LOW Ab–)), whereas CPEP-NORM comprised 163 patients (46/163 patients (28 percent) with islet antibodies (CPEP-NORM Ab+) and 117/163 patients (72 percent) without (CPEP-NORM Ab–)). Postglucagon C-peptide values of ≤0.60 nmol were found in all tested CPEP-LOW patients. Table 1 shows the clinical characteristics of the patients. CPEP-LOW patients were significantly younger at diagnosis, were slimmer, had higher insulin dosages, had lower stimulated C-peptide values, and were more often consid-

### Table 1. Relation of age, gender, diabetes duration, body mass index, insulin therapy, and clinical type of diabetes with C-peptide levels and islet autoantibody status among insulin-treated diabetic patients diagnosed in 1985–1991, Skaraborg County, Sweden, 1993–1996

<table>
<thead>
<tr>
<th></th>
<th>CPEP-LOW Ab+</th>
<th>CPEP-LOW Ab–</th>
<th>CPEP-NORM Ab+</th>
<th>CPEP-NORM Ab–</th>
<th>Controls</th>
<th>ANOVA+/* chi-square test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>111</td>
<td>71</td>
<td>46</td>
<td>117</td>
<td>216</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females (no. (%))</td>
<td>54 (48.6)</td>
<td>33 (46.5)</td>
<td>21 (46)</td>
<td>53 (45)</td>
<td>103 (47.7)</td>
<td>n.s.*</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis (years) (mean (SD))</td>
<td>24.5 (16.8)†</td>
<td>22.9 (19.0)†</td>
<td>43.9 (19.6)‡</td>
<td>53.5 (17.3)</td>
<td>29.8 (13.5)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Range (years)</td>
<td>2–77</td>
<td>1–77</td>
<td>5–82</td>
<td>5–81</td>
<td>15–81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;65 years (no. (%))</td>
<td>108 (97.3)§</td>
<td>68 (95.8)§</td>
<td>35 (76.1)</td>
<td>84 (71.8)</td>
<td>208 (96.35)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>&lt;30 years (no. (%))</td>
<td>73 (65.8)§</td>
<td>50 (70.4)§</td>
<td>16 (34.8)</td>
<td>16 (13.7)</td>
<td>133 (61.6)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Duration of diabetes (years) (mean (SD))</td>
<td>5.4 (2.3)</td>
<td>5.9 (2.1)</td>
<td>5.4 (1.8)</td>
<td>5.7 (2.0)</td>
<td>n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²) (mean (SD))</td>
<td>23.1 (3.9)§</td>
<td>22.9 (4.0)§</td>
<td>25.0 (3.3)§</td>
<td>28.5 (4.5)§</td>
<td>24.4 (3.6)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Insulin dose (IU/kg) (mean (SD))</td>
<td>0.76 (0.30)§§,¶</td>
<td>0.77 (0.29)§§,¶</td>
<td>0.58 (0.27)</td>
<td>0.52 (0.26)</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin treatment within 3 years after diagnosis (no. (%))</td>
<td>110 (99.1)†</td>
<td>65 (91.5)#</td>
<td>39 (84.8)‡</td>
<td>76 (67.5)</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting C-peptide (nmol/liter) (mean (SD))</td>
<td>0.12 (0.04)†</td>
<td>0.12 (0.04)†</td>
<td>0.47 (0.24)§</td>
<td>0.98 (0.56)</td>
<td>0.67 (0.26)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Range (nmol/liter)</td>
<td>&lt;0.10–0.60</td>
<td>&lt;0.10–0.20</td>
<td>0.30–1.60</td>
<td>0.30–2.80</td>
<td>0.30–1.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stimulated C-peptide (nmol/liter) (mean (SD))**</td>
<td>0.16 (0.10)§§,¶‡</td>
<td>0.19 (0.12)§§,¶‡</td>
<td>0.58 (0.24)§</td>
<td>1.49 (0.77)</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range (nmol/liter)</td>
<td>&lt;0.10–0.60</td>
<td>&lt;0.10–0.60</td>
<td>0.30–1.30</td>
<td>0.50–4.60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stimulated C-peptide (≤0.60 nmol/liter) (no. stimulated/total no. (%))</td>
<td>66/66 (100)†</td>
<td>41/41 (100)†</td>
<td>26/40 (65)</td>
<td>10/107 (9.3)</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical type 1 diabetes (no. (%))§§</td>
<td>94 (84.7)†</td>
<td>58 (81.7)§ §</td>
<td>22 (47.8)§</td>
<td>17 (14.5)</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* CPEP-LOW, patients with abnormally low C-peptide values, with (Ab+) and without (Ab–) islet autoantibodies; CPEP-NORM, patients with normal C-peptide values, with (Ab+) and without (Ab–) islet autoantibodies; ANOVA, analysis of variance (with post hoc) and chi-square test between patient groups; n.s., not significant; SD, standard deviation.
† Versus CPEP-NORM Ab+ and CPEP-NORM Ab–, p < 0.0001.
‡ Versus CPEP-NORM Ab+, p < 0.05.
§ Versus CPEP-NORM Ab+, p < 0.0001.
¶ Versus CPEP-NORM Ab–, p < 0.01.
# Versus CPEP-NORM Ab–, p < 0.001.
** Information on stimulated C-peptide (6 minutes postglucagon, 1 mg i.v.) was available for 254 patients.
†† Versus CPEP-NORM Ab+, p < 0.001.
‡‡ Versus CPEP-NORM Ab–, p < 0.05.
§§ As clinically classified by patients’ physicians.
TABLE 2. Distribution of HLA-DQA1-DQB1 genotypes in controls and among insulin-treated diabetic patients diagnosed in 1985–1991 subdivided into three groups according to C-peptide levels and islet autoantibody status, Skaraborg County, Sweden, 1993–1996

<table>
<thead>
<tr>
<th>DOA1-DQB1/DOA1-DQB1</th>
<th>CPEP-LOW*</th>
<th>CPEP-NORM Ab+*</th>
<th>CPEP-NORM Ab–*</th>
<th>Controls</th>
<th>2 × 4 contingency table p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>*0301-*0302/*0501-*0201</td>
<td>40</td>
<td>22.0†,§</td>
<td>6</td>
<td>13.0</td>
<td>8</td>
</tr>
<tr>
<td>*0301-*0302/X</td>
<td>66</td>
<td>36.3§,¶</td>
<td>16</td>
<td>34.8¶</td>
<td>12</td>
</tr>
<tr>
<td>*0501-*0201/X</td>
<td>36</td>
<td>19.8</td>
<td>7</td>
<td>15.2</td>
<td>25</td>
</tr>
<tr>
<td>*0102(3)-*0602(3)/X#</td>
<td>12</td>
<td>6.5§,**</td>
<td>3</td>
<td>6.5§,**</td>
<td>45</td>
</tr>
<tr>
<td>X/X</td>
<td>24</td>
<td>13.2††</td>
<td>14</td>
<td>30.5</td>
<td>27</td>
</tr>
<tr>
<td>MD*</td>
<td>4</td>
<td>2.2</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>All genotypes</td>
<td>182</td>
<td>100.0</td>
<td>46</td>
<td>100.0</td>
<td>117</td>
</tr>
</tbody>
</table>

* CPEP-LOW, patients with classic type 1 diabetes (low C-peptide levels with or without islet antibodies); CPEP-NORM, patients with normal C-peptide levels with (Ab+) or without (Ab–) islet antibodies; n.s., not significant; MD, missing data.
† Between-genotypes statistics, p values were corrected by a factor of five.
‡ Versus CPEP-NORM Ab–, p = 0.0025.
§ Versus controls, p < 0.0001.
¶ Versus CPEP-NORM Ab–, p < 0.0050.
# Distribution of the protective genotype *0102-*0602/X: CPEP-LOW, 1 (0.5%); CPEP-NORM Ab+, 0 (0.0%); CPEP-NORM Ab–, 25 (21.4%); controls, 61 (28.2%) with the same p values as in *0102(3)-*0602(3)/X.
** Versus CPEP-NORM Ab–, p < 0.0001.
†† Versus CPEP-NORM Ab+, p = 0.025.

CPEP-LOW patients

DOA1-DQB1 genotypes. Because there were no significant differences in the distribution of HLA-DQ genotypes between CPEP-LOW Ab+ patients and CPEP-LOW Ab– patients, these subgroups were merged into one single group (CPEP-LOW) representing classic type 1 diabetes. Table 2 demonstrates that the *0301-*0302/*0501-*0201 (OR = 10.42, 95 percent confidence interval (CI): 4.23, 25.67) and the *0301-*0302/X (OR = 5.29, 95 percent CI: 2.72, 10.29) type 1 diabetes high-risk genotypes were closely and positively associated with diabetes, whereas the DQA1*0102(3)-DQB1*0602(3)/X type 1 diabetes protective genotype was clearly negatively (OR = 0.31, 95 percent CI: 0.14, 0.68) associated with classic type 1 diabetes. The *0501-*0201/X genotype was, however, not associated with classic type 1 diabetes.

Table 3 shows that there were no significant associations between HLA-DQA1-DQB1 susceptibility and protective genotypes (and antibody status; data not shown) versus age at the time of diabetes diagnosis. Univariate analysis of variance revealed, however, a significant difference in age at diabetes diagnosis among high- and low-risk type 1 diabetes genotypes. A post hoc test showed that the 12 diabetic...
patients with the protective genotype *0102(3)-*0602(3)/X were significantly older (p < 0.05) at the time of diabetes diagnosis compared with the 40 patients with DQA1*0301-DQB1*0302/DQA1*0501-DQB1*0201 (33 years vs. 19 years). There was no association between gender and the different HLA genotypes (data not shown).

CPEP-NORM patients

Table 1 shows the clinical differences between CPEP-NORM Ab+ and Ab– patients. CPEP-NORM Ab+ patients had a significantly lower body mass index, an earlier need for insulin treatment, lower fasting and glucagon-stimulated C-peptide values, and a higher proportion of clinical type 1 diabetes than did CPEP-NORM Ab– patients.

DQA1-DQB1 genotypes. Table 2 shows that CPEP-NORM Ab+ patients had significantly higher frequencies of the DQA1*0301-DQB1*0302/X (35 percent vs. 12 percent) genotype and lower frequencies of the DQA1*0102(3)-DQB1*0602(3)/X (7 percent vs. 37 percent) genotype compared with controls. Hence, besides the DQA1*0301-DQB1*0302/DQA1*0501-DQB1*0201 genotype, HLA-DQ diabetes susceptibility and protective genotypes were similar in antibody-positive CPEP-NORM patients and in CPEP-LOW patients, that is, patients with classic type 1 diabetes. In contrast, table 2 shows that the HLA-DQ genotypes in CPEP-NORM Ab– patients were similar to those in controls; that is, CPEP-NORM Ab– patients seemed to have classic type 2 diabetes.

CPEP-NORM Ab+ patients

Table 4 shows that CPEP-NORM Ab+ patients could be divided into two groups (clinical type 1 diabetes and clinical type 2 diabetes, respectively) of equal sizes according to the clinical classification provided by the patient’s physician. There were no significant differences in insulin dose, fasting C-peptide levels, or postglucagon C-peptide levels between patients with clinical type 1 versus clinical type 2 diabetes. On the other hand, patients considered to have type 1 diabetes were significantly younger and had a lower body mass index than did those considered to have type 2 diabetes. Moreover, irrespective of clinical type 1 or type 2 diabetes, CPEP-NORM Ab+ patients had a significantly lower frequency of the type 1 diabetes protective *0102(3)-*602(3)/X compared with controls. Indeed, the *0102-*602/X was completely missing in both type 1 and type 2 CPEP-NORM Ab+ patients. In contrast, compared with controls, the type 1 diabetes risk genotypes *0301-*0302/*0501-*0201 and *0301-*0302/X were significantly increased in patients considered to have type 1 diabetes but not in those considered to have type 2 diabetes.

DISCUSSION

This study shows that, among insulin-treated diabetic patients with clear signs of type 1 diabetes (i.e., β-cell failure), there are no major effects of age at diagnosis on HLA associations. Classic type 1 diabetes seems to be uniform with respect to HLA genotypes. On the other hand, in patients with preserved β-cell function combined with islet autoimmunity, an interesting discrepancy in the HLA genotypes was observed. Islet autoimmunity was associated with a lack of HLA protective genotypes, whereas the clinical phenotype of type 1 diabetes was associated with HLA susceptibility genotypes. In contrast, patients with preserved β-cell function and without islet autoimmunity (classic type 2 diabetes) did not deviate with respect to HLA genotypes compared with controls. Hence, from the HLA perspective, patients with preserved β-cell function and islet autoimmunity seem to have characteristics closer to those of type 1 diabetes than those of type 2 diabetes.

Type 1 diabetes

The need to be treated with insulin to survive may be considered the classic definition of type 1 diabetes. In keeping with this, biochemical β-cell deficiency was considered the major criterion of type 1 diabetes in our study. An abnormally low fasting C-peptide value was therefore selected as a criterion for inclusion of patients in the CPEP-LOW group. The observation that all patients with an abnormally low fasting C-peptide concentration also fulfilled the classic criteria for insulin deficiency after the glucagon test (42) favors our classification of the patients into CPEP-LOW (most often type 1 diabetes) versus CPEP-NORM (most often type 2 diabetes) groups.

Using an abnormally low C-peptide value as the endpoint of type 1 diabetes, we could not detect any major effects of age at diagnosis on HLA-DQ susceptibility or protective genotypes associations in our population-based study of insulin-treated patients. Although a slight decrease of DQA1-DQB1 *0301-*0302/*0501-*0201 (susceptibility) and increase of *0102(3)-*0602(3)/X (protective) genotypes in patients over 35 years of age at diagnosis were observed, these differences were not significant. Accordingly, patients with type 1 diabetes seem to have a uniform type of diabetes with respect to HLA-DQ genotypes. Hence, with only about half of the patients tested in our study compared with that of Caillat-Zucman et al. (17), contrary to their findings, we could not confirm that the age at diagnosis of diabetes affects type 1 diabetes susceptible and protective HLA genotypes associations. Indeed, our results in Swedish patients are in agreement with the observation by Mizota et al. (48) in Japanese patients. It has, however, been reported that DQB1*0302 may predispose to occurrence of type 1 diabetes in young individuals, that is, patients 10 years of age or younger (10, 49). The number of patients in this age group was low in our study. The lack of an association between the *0301-*0302/X and *0102(3)-*0602(3)/X genotypes and age is in agreement with previous findings in 0- to 34-year-old Swedish (11) and Finnish (26) type 1 diabetic patients. Accordingly, we have to conclude that, on the basis of our study, the effect of age at diagnosis on HLA type 1 diabetes susceptibility and protective genotypes seems to be minimal or absent. Nevertheless, our observation that patients with the protective genotypes *0102(3)-*0602(3)/X were older at diagnosis of type 1 diabetes than patients with *0301-*0302/*0501-*0201 indicates that factors related to HLA genotypes are still of some importance for when type 1
HLA-DQ genotypes in Classic Type 1 Diabetes and in LADA

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Diabetes develops. This has also been stressed by Graham et al. (15) and Sabbah et al. (22), who found a decreasing frequency of DQA1-DQB1 *0501-*0201/*0301-*0302 and DQB1*20/*0302, respectively, with an increasing age at diagnosis of type 1 diabetes. In agreement with our observation that patients with *0102 (*3)-*0602 (*3)/X were older at diagnosis than other type 1 diabetic patients, these authors reported an increased frequency of DQA1-DQB1 *0102-*0602/X with age, suggesting an age-related attenuation of this genotype’s protective power.

The DQA1*0501-DQB1*0201/X genotype, a diabetes risk HLA genotype in some populations (50), was in our study neutral with respect to type 1 diabetes. Only in association with DQA1*0301-DQB1*0302 did DQA1*0501-DQB1*0201 confer the highest type 1 diabetes risk genotype, emphasizing that DQA1-DQB1 heterozygosity confers the highest type 1 diabetes risk, perhaps explained by the particularly diabetogenic heterodimers formed from gene products in trans (i.e., DQA1*0501 and DQB1*0302) (50, 51).

### Slow-progressing type 1 diabetes

We also tried to clarify whether there are patients with specific HLA genotypes associated with preserved β-cell function combined with islet autoantibodies (CPEP-NORM Ab+). Recently, it was suggested that genes associated with both type 1 and type 2 diabetes interact or operate separately in LADA (34). Indeed, this was confirmed in our study. Both patients with clinical type 1 and patients with clinical type 2 diabetes were found in our CPEP-NORM Ab+ group. Most

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<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>CPEP-NORM Ab+ (n = 46)</th>
<th>Clinical type 1 (n = 22)</th>
<th>Clinical type 2 (n = 24)</th>
<th>Controls (n = 216)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (no. (%))</td>
<td>15 (68.2)</td>
<td>10 (41.7)</td>
<td>113 (52.3)</td>
<td></td>
</tr>
<tr>
<td>Age (years) at diabetes onset or sampling of controls (mean (SD)*))</td>
<td>26.6 (14.9)†</td>
<td>59.7 (14.5)†</td>
<td>29.8 (13.5)</td>
<td></td>
</tr>
<tr>
<td>&lt;65 years of age (no. (%))</td>
<td>21 (95.5)‡</td>
<td>15 (62.5)‡</td>
<td>208 (96.4)</td>
<td></td>
</tr>
<tr>
<td>Diabetes duration (years) (mean (SD))</td>
<td>4.8 (1.7)§</td>
<td>5.9 (1.8)§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²) (mean (SD))</td>
<td>23.3 (2.0)¶</td>
<td>26.4 (3.6)¶</td>
<td>24.4 (3.6) ¶</td>
<td></td>
</tr>
<tr>
<td>Insulin dose (IU/kg) (mean (SD))</td>
<td>0.64 (0.32)</td>
<td>0.52 (0.21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin within 3 years (no. (%))</td>
<td>22 (100.0)</td>
<td>17 (70.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting C-peptide (nmol/liter) (mean (SD))</td>
<td>0.44 (0.17)</td>
<td>0.50 (0.29)</td>
<td>0.67 (0.26)</td>
<td></td>
</tr>
<tr>
<td>Postglucagon C-peptide (nmol/liter) (mean (SD)), (n = 20 and 20, respectively)</td>
<td>0.58 (0.23)</td>
<td>0.59 (0.27)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* CPEP-NORM Ab+, patients with normal C-peptide levels with (Ab+) islet antibodies; SD, standard deviation; MD, missing data.
† Clinical characteristics (Mann-Whitney), clinical type 1 versus type 2, p < 0.0001.
‡ Clinical characteristics, clinical type 1 versus type 2, p < 0.01 (chi-square test).
§ Clinical characteristics (Mann-Whitney), clinical type 1 versus type 2, p = 0.0454.
¶ Clinical characteristics (Mann-Whitney), clinical type 1 versus type 2, p = 0.0040.
# DQA1-DQB1 genotypes (chi-square test or Fisher’s exact test): clinical type 1 versus controls, p = 0.017.
** DQA1-DQB1 genotypes (Fisher’s exact test): clinical type 1 versus controls, p < 0.0001.
†† The protective genotype *0102-*0602/X was absent in both clinical types (significantly lower frequencies as compared with the 61 (28.2%) controls versus type 1, p = 0.0075; versus type 2, p = 0.0045).
‡‡ DQA1-DQB1 genotypes (chi-square test): clinical type 1 versus controls, p = 0.0075.
§§ Clinical type 1 versus type 2, all genotypes: not significant; clinical type 2 versus controls, p = 0.0265.
Interestingly, among antibody-positive patients with preserved β-cell function (CPEP-NORM Ab+), clinical type 1 diabetes was strongly associated with the HLA susceptibility genotypes, whereas such associations were lacking in corresponding patients with clinical type 2 diabetes. Hence, both patients with and patients without type 1 diabetes susceptibility genotypes were found in this group of patients (CPEP-NORM Ab+). Moreover, unrelated to the phenotype of diabetes and the HLA diabetes susceptibility genotypes, lack of the HLA diabetes protective genotypes *0102(3)*-0602(3)/X was associated with islet autoimmunity in our patients suspected to have LADA. This observation is consistent with those of Pugliese et al. (7, 52), who found islet autoimmunity in relatives of type 1 diabetic patients to be unassociated with the development of type 1 diabetes in the presence of DQB1*0602.

HLA studies in LADA patients are rare. It has been suggested that the typical HLA genetic predisposition to type 1 diabetes is less marked in LADA than in patients diagnosed at a younger age (53). In agreement with this concept but in contrast to our study, Tuomi et al. (26) reported that LADA patients (defined as glutamic acid decarboxylase antibody-positive type 2 diabetes patients) had a higher frequency (38 percent) of protective HLA-DQB1*0602(3) compared with type 1 diabetic patients (16 percent). Indeed, also in contrast to our findings, in CPEP-NORM Ab+ patients with clinical type 2 diabetes, HLA susceptibility genotypes DQB1*0201/DQB1*0302 and DQB1*0302/X were found with increased frequency in the LADA patients of Tuomi et al. compared with their controls (35 percent vs. 16 percent). How can this discrepancy be explained? An obvious possibility is differences in patient populations. Tuomi et al. investigated patients with a known diagnosis of type 2 diabetes. On the basis of their observations, Tuomi et al. defined LADA as glutamic acid decarboxylase antibody-positive clinical type 2 diabetic patients above the age of 35 years at diagnosis without a need for insulin treatment within 6 months, whereas our patients had preserved β-cell function after 6 years of diabetes. Because many islet antibody-positive adult diabetic patients develop β-cell failure within 5 years after diagnosis (28), it is possible that classic type 1 diabetic patients were included in the study by Tuomi et al. In our study, classic type 1 diabetic patients were found in the CPEP-LOW group. Another possibility is differences between Swedish and Finnish (Bothnic) populations as supported by Ilonen et al. (54), who actually found differences in DQB1*0301 versus DQB1*0302 frequencies between different subpopulations in Finland.

The possibility that LADA is found in a heterogeneous group of patients also has to be considered. In the classic description of LADA, islet autoimmunity is associated with impaired β-cell function in type 2 diabetic patients (25, 31, 32). In contrast, the CPEP-NORM Ab+ patients showed preserved β-cell function 6 years after diagnosis of diabetes. Moreover, among CPEP-NORM Ab+ patients, those with clinical type 1 diabetes were younger at diagnosis and were immediately put on insulin, whereas those with clinical type 2 diabetes had been treated with oral hypoglycemic agents alone for more than 2 years. It is now clear that, in slow-progressing type 1 diabetes (LADA), autoimmunity is not latent and not limited to adults (33). Although insulin treatment in our LADA patients may have prevented β-cell failure (55), only prospective observation of our CPEP-NORM Ab+ patients will reveal whether proven islet autoimmunity is associated with future β-cell failure. Until then, our study suggests that insulin-treated LADA patients, rather, have a lack of HLA protective genotypes than a presence of HLA diabetes susceptibility genes. It could be that, among our CPEP-NORM Ab+ patients, only those with HLA susceptibility genes will develop β-cell failure and overt type 1 diabetes in the future. Future prospective observation of our patients will clarify this issue.

Conclusion

Our study did not find any major effect of age on HLA-DQ associations in classic type 1 diabetes. Among insulin-treated diabetic patients with islet antibodies and preserved β-cell function (LADA), a clear negative association with HLA-DQ protective genotypes was detected, whereas HLA-DQ type 1 diabetes susceptibility genes were not mandatory.

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