Geographical Variation in Risk HLA-DQB1 Genotypes for Type 1 Diabetes and Signs of β-Cell Autoimmunity in a High-Incidence Country

Marika Kukko, MD1,2
Sari Korhonen, MD1,6
Juventude Diabetes Research Foundation Center for the Prevention of Type 1 Diabetes in Finland, Tampere, Finland; the 1Department of Pediatrics, University of Oulu, Oulu, Finland; the 2Department of Virology, University of Turku, Turku, Finland; the 3Department of Pediatrics, University of Turku, Turku, Finland; the 4Department of Public Health Institute, Helsinki, Finland; the 5Tampere School of Public Health, University of Tampere, Finland; the 6Department of Epidemiology and Health Promotion, National Public Health Institute, Helsinki, Finland; the 7Department of Epidemiology and Health Promotion, National Public Health Institute, Helsinki, Finland; the 8Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland.

OBJECTIVE — To assess possible differences in the frequency of HLA-DQB1 risk genotypes and the emergence of signs of β-cell autoimmunity among three geographical regions in Finland.

RESEARCH DESIGN AND METHODS — The series comprised 4,642 children with increased HLA-DQB1–defined genetic risk of type 1 diabetes from the Diabetes Prediction and Prevention (DIPP) study: 1,793 (38.6%) born in Turku, 1,646 (35.5%) in Oulu, and 1,203 (25.9%) in Tampere. These children were examined frequently for the emergence of signs of β-cell autoimmunity, for the primary screening of which islet cell antibodies (ICA) were used. If the child developed ICA, all samples were also analyzed for insulin autoantibodies (IAA), GAD65 antibodies (GADA), and antibodies to the IA-2 molecule (IA-2A).

RESULTS — The high- and moderate-risk genotypes were unevenly distributed among the three areas (P < 0.001); the high-risk genotype was less frequent in the Oulu region (20.4%) than in the Turku (28.4%; P < 0.001) or Tampere regions (27.2%; P < 0.001). This genotype was associated with an increased frequency of ICA seroconversion relative to the moderate risk genotypes (hazard ratio 1.89, 95% CI 1.36–2.62). Seroconversions to ICA positivity occurred less commonly in Tampere than in Turku (0.47, 0.28–0.75), whereas the seroconversion rate in Oulu did not differ from that in Turku (0.72, 0.51–1.03). The Tampere-Turku difference persisted after adjustment for risk genotypes, sex, and time of birth (before January 1998 versus later). Seroconversion for at least one additional autoantibody was also less frequent in Tampere than in Turku (0.39, 0.16–0.82).

CONCLUSIONS — These data show that in Finland, the country with the highest incidence of type 1 diabetes in the world, both the frequency of the high-risk HLA-DQB1 genotype and the risk of seroconversion to autoantibody positivity show geographical variation. The difference in seroconversion rate could not be explained by the difference in HLA-DQB1–defined disease susceptibility, implying that the impact of environmental triggers of diabetes-associated autoimmunity may differ between the three regions studied.

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land (12,13). In China, where the incidence rate is one of the lowest reported, a 12-fold geographical variation has been observed (14). Regional variation was also reported in Finland during the late 1980s (15), with persistently high-risk areas located in central Finland from 1987 to 1996, although the geographical pattern changed with time (16). It has also been suggested that autoimmunity in first-degree relatives of patients with type 1 diabetes occurs with similar frequencies in different European countries despite the wide variation in the incidence of the clinical disease (17). However, when the prevalence of B-cell autoimmunity and the incidence of type 1 diabetes in the background population were compared in eight European countries, a relatively close correlation was found between the two parameters (18).

Because diabetes-associated autoantibodies, particularly islet cell antibodies (ICAs), insulin autoantibodies (IAAs), GAD65 antibodies (GADAs), and antibodies to the IA-2 molecule (IA-2As), markedly increase the risk of progression to type 1 diabetes in subjects carrying HLA-DQ susceptibility alleles, we decided to assess whether the prevalence of the HLA-DQB1 risk genotypes differs among the three regions of Finland (Tampere, Turku, Oulu) covered by the DIPP study and whether ICA alone or in combination with other diabetes-associated autoantibodies emerge at different rates in these three regions (Fig. 1).

**RESEARCH DESIGN AND METHODS** — Screening of HLA-DQ risk alleles in newborn infants was initiated at the Turku University Hospital in November 1994, at the Oulu University Hospital in September 1995, and at the Tampere University Hospital in October 1997. Cord blood samples were obtained from all newborn infants, and the families received oral and written information on type 1 diabetes and the DIPP study. More than 94% of the families gave their written informed consent to the genetic screening. Families in which neither of the parents was of Caucasian origin (>99% of the Finnish population are Caucasian), in which the parents had difficulty understanding Finnish, Swedish, or English, or in which the newborn infant had a severe congenital disease were excluded from the study. During the time period between November 1994 and November 2000, 47,605 infants were screened for HLA-DQB1–conferred genetic risk. Families with an infant carrying increased HLA-conferred susceptibility to type 1 diabetes (n = 5,978; 12.6% of all screened) were invited for observation and immunological surveillance for the emergence of diabetes-associated autoantibodies and development of type 1 diabetes. The present series comprised 4,642 such children (77.7% of those invited) from the three regions of Finland, of whom 52.7% (2,447) were boys.

Serum samples for the immunological surveillance were taken at the ages of 3, 6, 12, 18, 24, and 36 months, and the present analysis is based on samples taken before the end of March 2001. The protocol was approved by the ethical committees of the three participating hospitals, and informed consent was obtained from the parents or guardians of the children.

**Genetic screening**

HLA-DQB1 typing was performed by a previously described method based on time-resolved fluorescence (19). Five sequence-specific oligonucleotide probes were used to identify the following DQB1 alleles known to be significantly associated with either susceptibility to or protection against type 1 diabetes in the Finnish population: DQB1*0302, DQB1*02, DQB1*0602, DQB1*0603, and DQB1*0301. The subjects were classified into four risk groups based on their HLA-DQB1 genotype using a previously described simplified classification: high risk (DQB1*02/0302), moderate risk (DQB1*0302/x, where x indicates *0302 or a nondefined allele), low risk (DQB1*0301/0302, DQB1*02/0301, DQB1*02/x, DQB1*0302/0602; where x indicates *02 or a nondefined allele), and decreased risk (protection) (DQB1*x/x, DQB1*0301/x, DQB1*02/0602, DQB1*02/0603, DQB1*0602/x, DQB1*0603/x, where x indicates a nondefined allele) (20). Subjects carrying the HLA-DQB1*0302/0603 genotype were
Within-country differences in β-cell autoimmunity

Table 1—ICA positivity, multiple autoantibody positivity (ICA plus at least one other autoantibody), and length of follow-up of the subjects by region of residence (for details, see Research Design and Methods)

<table>
<thead>
<tr>
<th></th>
<th>Turku</th>
<th>Oulu</th>
<th>Tampere</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>1,793</td>
<td>1,646</td>
<td>1,203</td>
</tr>
<tr>
<td>ICA positive</td>
<td>81 (4.5)</td>
<td>49 (3.0)</td>
<td>20 (1.7)</td>
</tr>
<tr>
<td>High risk</td>
<td>34 (1.9)</td>
<td>16 (1.0)</td>
<td>9 (0.7)</td>
</tr>
<tr>
<td>Moderate risk</td>
<td>47 (2.6)</td>
<td>33 (2.0)</td>
<td>11 (0.9)</td>
</tr>
<tr>
<td>Multiple antibodies</td>
<td>35 (2.0)</td>
<td>25 (1.5)</td>
<td>7 (0.6)</td>
</tr>
<tr>
<td>Follow-up (months)</td>
<td>24.0 (1.5–36)</td>
<td>24.0 (1.5–36)</td>
<td>12.0 (1.5–36)</td>
</tr>
</tbody>
</table>

Data are n (%) or median (range).

Antibody analyses

The diabetes-associated autoantibodies were analyzed in the Research Laboratory, Department of Pediatrics, University of Oulu. ICAs were used as the initial screening test for β-cell autoimmunity. If a child tested positive for ICA, all samples drawn from that individual were studied for IAA, GADA, and IA-2A. ICAs were used as the initial screening test for Tampere (23) and for Oulu (21), the antibodies being determined in a disease sensitivity of 42% and specificity of 100% in a blinded reanalysis of the 100 workshop samples. The samples initially analyzed for IAA were retested with the more sensitive assay.

GADAs were measured with a radioassay as described (26). The results were expressed in RU based on a standard curve constructed from a dilution of positive and negative samples. The cutoff limit for antibody positivity (5.36 RU) was set at the 99th percentile in 373 nondiabetic Finnish children and adolescents. The disease sensitivity of the GADA assay was 76% and its specificity was 96%, based on the 2001 DASP workshop (25).

IA-2As were quantified with a radioassay as previously described (27); antibody values were expressed in RU based on a standard curve, as for GADs. The limit for antibody positivity was set at 0.43 RU, which represents the 99th percentile in 374 nondiabetic Finnish children and adolescents. The disease sensitivity of this assay was 58% and the specificity was 100%, based on the 1999 DASP workshop (25). Samples with an IAA, GADA, or IA-2A value between the 97.5th and 99.5th percentiles were reanalyzed to confirm their antibody status. Maternal antibodies, which were present in cord blood and thereafter decreased and disappeared from the child’s serum at the latest by 15 months of age (28), were excluded from the analyses.

Statistical analyses

To account for the interval censoring caused by the discrete time points of the measurements, the hazard ratio for seroconversion to positivity for type 1 diabetes–associated autoantibodies (ICA, other autoantibodies in addition to ICA) during the 3-year follow-up period was estimated by life-table survival regression in relation to region of residence, sex, and genotype (29). Two binary variables were used for region (Tampere versus Turku and Oulu versus Turku). Possible differences in the prevalence of the risk genotypes among the regions were sought by means of cross-tabulation and the χ² test. A two-sided P value <0.05 was considered significant.

RESULTS—The median follow-up time in this series was 21.0 months (range 1.5–36, Table 1). Approximately one-fourth of the 4,642 children (1,172; 25.2%) had the high-risk genotype and 74.8% (3,470) had moderate-risk genotypes, whereas 3.2% (150) gave an ICA-positive sample on at least one occasion during the follow-up. The high- and moderate-risk genotypes were unevenly distributed among the university hospital regions (P < 0.001); the Turku and Tampere regions had similar proportions of the high- and moderate-risk genotypes (P = 1.00), whereas Oulu had a higher proportion of children with moderate-risk genotypes and fewer with the high-risk genotype than did Turku (P < 0.001) or Tampere (P < 0.001) (Fig. 2).

Residence in the Tampere region was associated with a reduced rate of seroconversion to ICA positivity. This difference remained significant even when the HLA-DQB1 genotype was taken into account (Table 2A). There were significant differences between Tampere and Turku among those with the moderate-risk genotypes (Fig. 3A and B). A conspicuous feature was that not a single high-risk sub-

excluded from the present study cohort, because they were initially excluded but later included in the series invited for regular surveillance. Of the 15,507 infants screened in Oulu, 416 (2.7%) carried the high-risk genotype, 1,673 (10.8%) carried moderate-risk genotypes, 3,464 (22.3%) carried low-risk genotypes, and 9,974 (64.2%) carried high-risk genotypes, 1,673 (10.8%) carried moderate-risk genotypes, 3,464 (22.3%) carried low-risk genotypes, and 9,974 (64.2%) carried genotypes conferring decreased risk. The corresponding numbers among the 11,591 subjects screened in Tampere were 380 (3.3%), 1,060 (9.2%), 2,814 (24.3%), and 7,337 (63.3%). There were 650 infants (3.2%) with the high-risk genotype, 1,799 (8.8%) with moderate-risk genotypes, 5,114 (24.9%) with low-risk genotypes, and 12,944 (63.1%) with genotypes associated with decreased risk among the 20,507 children screened in Turku.
ject seroconverted to ICA positivity during the first year of life in Tampere. The sex of the child was not associated with seroconversion to ICA positivity when adjusted for genotype (boys versus girls, hazard ratio 1.18, 95% CI 0.86–1.63), neither was the time of birth (before January 1998 versus later) related to the seroconversion rate when adjusted for area and genetic risk (1.13, 0.79–1.61). Positivity for multiple autoantibodies, i.e., ICA and at least one other autoantibody (IAA, GADA, and/or IA-2A), was also less common in Tampere (Table 2B). The differences between the two regions could be seen clearly among those with the high-risk genotype (P = 0.025), whereas there were no regional differences between the children carrying moderate-risk genotypes (P = 0.40).

Close to 4% of the total cohort (171 of 4,642; 3.7%) had at least one family member affected by type 1 diabetes. The frequency of familial diabetes was 4.1% in Turku (n = 74), 2.9% in Oulu (n = 48), and 4.1% in Tampere (n = 49; P = 0.12). Among the children with an affected family member, 7.0% (12 of 171) had at least ICA, whereas the corresponding proportion was 3.1% (138 of 4,471) among those without any affected first-degree relative (P = 0.004).

CONCLUSIONS — The DIPP project was launched in 1994 in Finland, the country with the highest incidence of type 1 diabetes in the world, to define optimal methods of identifying children with increased risk of type 1 diabetes in the general population and to develop effective tools for preventing or delaying progression to clinical disease. In this study, the data collected from the University Hospitals in Turku, Oulu, and Tampere are generally processed and analyzed as one dataset. In the present study, we were interested in whether the occurrence of HLA-DQB1 alleles and the appearance of signs of β-cell autoimmunity would differ among these three geographical regions. Positivity for ICA was used as a primary sign of β-cell autoimmunity. If a child became positive for ICA, all other samples were analyzed for the appearance of any of the other three autoantibodies. The positive predictive values of ICA, IA-2A, GADA, and IAA among siblings of the affected children in the Childhood Diabetes in Finland (DiMe) study were found to be 43, 55, 42, and 29% and the sensitivities were 81, 69, 69, and 25%, respectively, over an 8-year follow-up period, implying that IA-2A had the highest positive predictive value and ICA had the highest sensitivity for type 1 diabetes (30). In a recent study, based on the analysis of all four autoantibodies in a subgroup of 1,005 DIPP children, we showed that ICA had the highest sensitivity and specificity for persistent positivity for at least two diabetes-associated autoantibodies (31), which can be regarded as a strong predictive surrogate marker of clinical type 1 diabetes (28). Our current data show that the risk of seroconversion to ICA positivity was highest in the Turku region and lowest in the Tampere area. The reduced frequency of ICA positivity observed in the Oulu region relative to Turku became nonsignificant after adjustment for HLA genotypes. Our results confirm that the high-risk HLA genotype is more closely associated with ICA positivity than the moderate-risk genotypes.

Because it is apparent that positivity for multiple (≥2) autoantibodies is associated with a markedly increased risk of progression to clinical disease in first-degree relatives of patients with type 1 diabetes (32), it was of interest to assess whether the incidence of multiple autoantibodies was different among the regions studied here. These analyses gave results similar to those observed for ICA, as the children in the Tampere region also had multiple autoantibodies less often than age-matched children in the Turku area.

Table 2—Hazard ratios and 95% CIs of seroconversion to ICA positivity (A) and having at least one other autoantibody in addition to ICA (B) associated with living in the region of the Turku, Oulu, or Tampere University Hospitals and associated with HLA-DQB1 genotype

<table>
<thead>
<tr>
<th>Region/Genotype</th>
<th>Unadjusted hazard ratio</th>
<th>95% CI</th>
<th>P value</th>
<th>Adjusted* hazard ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) Region</td>
<td></td>
<td></td>
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<tr>
<td>Tampere versus Turku</td>
<td>0.53</td>
<td>0.32–0.86</td>
<td>0.008</td>
<td>0.54</td>
<td>0.32–0.87</td>
<td>0.010</td>
</tr>
<tr>
<td>Oulu versus Turku</td>
<td>0.70</td>
<td>0.49–0.99</td>
<td>0.044</td>
<td>0.74</td>
<td>0.51–1.05</td>
<td>0.092</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High versus moderate risk</td>
<td>1.94</td>
<td>1.39–2.68</td>
<td>&lt;0.001</td>
<td>1.90</td>
<td>1.36–2.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B) Region</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tampere versus Turku</td>
<td>0.42</td>
<td>0.17–0.88</td>
<td>0.021</td>
<td>0.42</td>
<td>0.17–0.90</td>
<td>0.024</td>
</tr>
<tr>
<td>Oulu versus Turku</td>
<td>0.83</td>
<td>0.49–1.38</td>
<td>0.471</td>
<td>0.92</td>
<td>0.54–1.53</td>
<td>0.738</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High versus moderate risk</td>
<td>2.89</td>
<td>1.78–4.67</td>
<td>&lt;0.001</td>
<td>2.89</td>
<td>1.78–4.69</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Adjusted for all the variables in the table.
The Finnish population is considered fairly homogeneous and has been relatively isolated for many centuries (33). Still, there are geographical differences in the prevalences of HLA genotypes and alleles within the country. The National Bone Marrow Registry has also been used to study HLA allele frequencies in the Finnish population, and substantial regional variations were seen in allele frequencies among different geographical regions of Finland (34). A difference in type 1 diabetes–associated HLA-DQB1 genotypes has been reported earlier between southwest (Turku) and northern (Oulu) parts of the country, based on the DIPP study cohort (6). In the present work, Tampere was also included in the analysis. The present findings confirm the previous observation, in that the proportion of high-risk children was significantly lower in the Oulu region than in Turku or Tampere.

In summary, our results show that there is geographical variation both in the frequency of the HLA-DQB1 genotype, which is associated with the greatest susceptibility to type 1 diabetes, and in the risk of seroconversion to ICA positivity or positivity for multiple autoantibodies. The latter differences were not explained by HLA-defined disease susceptibility.

Furthermore, the children in Tampere had first-degree relatives affected by type 1 diabetes as often as did those in Turku, so that this could not explain why a higher proportion seroconverted in Turku. These findings could be explained by environmental factors, possibly caused by differences in climate between Turku and Tampere, and exercising their effect via associated differences in the pattern of certain infections. Turku is located in the southern part of the country by the sea, and the climate is more temperate than in Tampere, especially in winter. Another explanation may be that allele frequencies in other type 1 diabetes genetic susceptibility loci, either within or outside the HLA region of chromosome 6, may differ among regions. It remains to be seen in later studies whether the differences in the appearance of β-cell autoimmunity in this population also result in similar differences in the presentation of clinical disease.

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