CLINICAL STUDY

Extended family history of autoimmune diseases and phenotype and genotype of children with newly diagnosed type 1 diabetes

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Abstract

Objective: Based on the concept of clustering autoimmunity, children with a positive family history of autoimmunity could be expected to have a different pathogenetic form of type 1 diabetes (T1D) and thus a stronger autoimmune reactivity against β -cells and an increased prevalence of the HLA-DR3-DQ2 haplotype.

Design and methods: We tested this hypothesis in a cross-sectional observational study from the Finnish Pediatric Diabetes Register. HLA class II genotypes and β -cell autoantibodies were analyzed, and data on the extended family history of autoimmunity and clinical markers at diagnosis were collected with a structured questionnaire from 1488 children diagnosed with T1D under the age of 15 years (57% males).

Results: Only 23 children (1.5%) had another autoimmune disease (AID) known at diagnosis, and they had a milder metabolic decompensation at diabetes presentation. One-third (31.4%) had at least one relative with an AID other than T1D with affected mothers being overrepresented (8.2%) compared with fathers (2.8%). The children with a positive family history of other AIDs had higher levels of islet cell antibodies ($P=0.003$), and the HLA-DR3-DQ2 haplotype in the children was associated with celiac disease in the extended family ($P<0.001$), but not with an increased frequency of autoimmune disorders, in general.

Conclusions: Approximately one-third of children with newly diagnosed T1D have a first- and/or second-degree relative affected by an AID. Our data do not consistently support the hypothesis of differential pathogenetic mechanisms in such children.

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Introduction

Autoimmunity clusters in individuals and in families are due to largely unknown genetic and environmental factors. Type 1 diabetes (T1D) is associated with other autoimmune disorders such as autoimmune thyroiditis (AIT) (1, 2, 3, 4, 5), celiac disease (CD) (1, 3, 4, 5), Addison's disease (2, 4, 5), pernicious anemia (4, 5), rheumatoid arthritis (1, 4), and multiple sclerosis (6, 7). Increased risk of multiple autoimmune manifestations already exists at T1D diagnosis, but it increases significantly with follow-up (8, 9). At diagnosis, 9–19% of the children with T1D have another autoimmune disease (AID) based on autoantibody screening (8, 10); CD (1.5–3.3%) (10, 11, 12, 13) and AIT (0.6–3.1%) (10, 13, 14) are the most common conditions. Relatives of patients are at a greater risk

of AIDs with AIT, CD, and rheumatoid arthritis as the most common conditions (1, 3, 8, 15, 16, 17, 18).

The HLA class II haplotypes *DRB1*0401/2/4/5-DQA1*0301-DQB1*0302* (DR4-DQ8) and (*DRB1*03-DQA1*05-DQB1*02*) (DR3-DQ2) are the major contributors to the genetic risk of T1D among Caucasians, and the latter also strongly predisposes to CD and other AIDs (11, 19, 20, 21, 22, 23, 24, 25). Among the T1D-related autoantibodies, glutamic acid decarboxylase autoantibodies (GADAs) have been directly associated with (14, 20, 22, 26) and antibodies to the islet antigen 2 protein (IA-2A) have been inversely associated (20) with the risk of other AIDs.

Studies evaluating the frequency of other AIDs in the extended family from the time of diagnosis of T1D are scarce, and the effects of a positive family history of AIDs on the phenotype and genotype of newly diagnosed

children are largely unknown. We, therefore, set out to characterize the effects of a positive history of other AIDs in the extended family on metabolic, immunological, and genetic markers in children with newly diagnosed T1D. We hypothesized that the increased burden of autoimmunity would lead to a pathogenetically distinct subset of T1D in children with a positive family history of other AIDs. These children were expected to have an increased prevalence of the DR3-DQ2 haplotype and a stronger reactivity against β -cell antigens, reflected by more frequent autoantibodies and higher titers.

Subjects and methods

Study design and subjects

The nationwide Finnish Pediatric Diabetes Register (27) has covered more than 90% of children diagnosed with T1D since June 2002 (28). By April 2007, the register had covered 1544 children who had T1D-related autoantibodies analyzed and were diagnosed with T1D before the age of 15 years. Children with no information on their relatives in the register and one child with a known insulin gene mutation were excluded. Only one child from each family was included as the index case. Thus, the study cohort comprised 1488 children with a median age of 8.23 years (range 0.28–14.99 years) at diagnosis, and the proportion of boys was 56.9%. Serum samples were obtained at a median of 5 days after diagnosis.

The register contains information on the family history of AIDs collected by a structured questionnaire (29). The families are asked to list any family members with AIDs, and the following examples are given: CD, dermatitis herpetiformis, AIT, autoimmune adrenal dysfunction, rheumatoid arthritis, multiple sclerosis, pernicious anemia, and systemic lupus erythematosus. They are asked about the total number of first-degree relatives (parents and siblings), but not about that of second-degree relatives (grandparents and siblings of parents). The register does not include follow-up of the families after the diagnosis.

Approximately 70% of the families participating in the register also provided blood samples for the Biobank. T1D-related autoantibodies (islet cell antibodies (ICAs), insulin autoantibodies (IAAs), GADA, and IA-2A) and *HLA-DR-DQA1-DQB1* haplotypes (30) were analyzed. Legal guardians and every subject aged 18 years or above gave written informed consent. Participants aged 10–17 years gave written assent. The Ethics Committee of the Hospital District of Helsinki and Uusimaa approved the protocol.

For the analysis, different groupings were applied. First, the children with a known additional AID already at diagnosis were compared with those with T1D only. Second, a few descriptions of a positive family history of AIDs were used: the children with first- and/or second-degree relatives (extended family) with AID diagnoses, the children with a positive extended family

history specifically of AIT (hypo/hyperthyroidism), CD (CD, dermatitis herpetiformis), or rheumatoid diseases (e.g. rheumatoid arthritis and systemic lupus erythematosus, Sjögren's syndrome, ankylosing spondylitis, mixed connective tissue disease, and scleroderma), and the children belonging to the so-called autoimmune families (extended families with greater than three AID diagnoses (different diseases and/or family members, e.g. two persons both of whom have T1D and CD) and/or greater than two different AIDs including diagnoses of the index child). These groups were then compared with children with no family history of T1D or any other AIDs.

To allow the observation of the effects of a positive family history of only AIDs other than T1D, children with a positive family history of T1D were excluded from the analysis, except when defining autoimmune families and analyzing index children with an additional AID or T1D only. The families who did not provide any information on AIDs of any family members (10/1488) were counted as not having any family members affected by AIDs. For the rest of the families, we included all the provided information in the analysis even when the information on the extended family was incomplete.

Autoantibody assays

IAA, GADA, and IA-2A levels were quantified with specific radiobinding assays (31, 32, 33) with cutoff limits of 2.80, 5.36, and 0.77 relative units (RU) respectively. The limits for positivity were based on the 99th percentiles in more than 350 Finnish control children. In the 2009 Diabetes Autoantibody Standardization Program (DASP), these assays exhibited sensitivities of 42, 78, and 64% and specificities of 99, 95, and 99% respectively. ICAs were analyzed with indirect immunofluorescence using human group 0 donor pancreas and expressed in Juvenile Diabetes Foundation (JDF) units with 2.5 JDF units as the detection limit (34). We included only results at or above the cutoffs for the calculation of median titers.

HLA genotyping

We used a PCR-based lanthanide-labeled hybridization method using time-resolved fluorometry for the detection of the major T1D risk-associated DR-DQ haplotypes (30). The number of children with HLA typing available was 1454 (97.7%).

Markers of metabolic decompensation

Local laboratories analyzed plasma glucose and β -hydroxybutyrate levels and pH at diagnosis. Data on plasma glucose levels were missing in 19 subjects (1.3%), on pH in 35 subjects (2.4%), and on β -hydroxybutyrate in 284 subjects (19.1%). Owing to

Figure 1 Grouping of the index cases according to who in the extended family is affected by AIDs. The dashed lines indicate cases with affected relatives from more than one category of relatives. These cases are also included in the total number of each category of relatives. AID, autoimmune disease (other than T1D); T1D, type 1 diabetes.

a lack of nationwide standardization, HbA1c measurements were not recorded.

Statistical analysis

IBM SPSS 19 statistical software package and R 12.2.1 package for statistical computing (35) were used for the analysis. For the comparison of frequencies, cross-tabulation, χ^2 -statistics, and Fisher's exact test were used. Continuous variables with a normal distribution were analyzed with Student's *t*-test and those with a skewed distribution with Mann–Whitney *U* test/Wilcoxon's rank sum test. Logistic regression and quantile regression in R (quantreg 4.54) were used when adjusting for confounding factors. A two-tailed *P* value of <0.05 was considered statistically significant. Bonferroni's correction for multiple comparisons was not applied due to its overly conservative nature. Multiplicity issues were taken into account in cautious interpretation of the results.

Results

History of AIDs

The number of children with another AID already known at T1D diagnosis was 23 (1.5%); CD was the most common condition (10/23), followed by AIT

(9/23), rheumatoid arthritis (4/23), and colitis ulcerosa (1/23). One child had both AIT and rheumatoid arthritis. Considering the family history of AIDs, a total of 467 index children (31.4%) had at least one extended family member with an AID other than T1D (Fig. 1). At least one affected first-degree relative was reported by 167 (11.2%) cases and an affected second-degree relative by 357 cases (24.0%) and 57 cases (3.8%) had both. More children had an affected mother (8.1%) than an affected father (2.8%; $P<0.001$), and 13.0% of the children had an affected maternal grandparent and 9.1% had an affected paternal grandparent ($P<0.001$). An affected maternal relative was reported by 16.0% of the cases and an affected paternal relative by 11.2% ($P<0.001$). The number of index cases with an affected sibling was 13 (0.9%), which is 1.1% of those 1200 index cases who had siblings. The prevalence of an AID other than T1D was 0.7% among all the siblings (14/2087) and 5.8% among the grandparents (343/5952).

The number of autoimmune diagnoses in the extended family, including diseases of the index child and T1D, varied between 1 and 13 and the number of different AIDs varied between 1 and 5. When T1D was excluded, the figures were 0–8 and 0–4 respectively. According to these data, 150 (10.1%) subjects fulfilled our criteria for an autoimmune family (Fig. 1). AIT in the extended family was reported by 17.0% of the families, rheumatoid disease by 12.0%, CD by 5.2%, and other AIDs by 3.5%. These diseases were more frequently reported in second-degree relatives than in first-degree ones (Fig. 2).

The sex distribution and the age at diagnosis of the index cases were similar in the groups of comparison, although the children with a known additional AID tended to be older (Table 1). The children with relatives affected by an AID came, in general, from larger

Figure 2 Proportions of children with newly diagnosed type 1 diabetes (T1D) with first- or second-degree relatives affected by autoimmune diseases other than T1D (white bars indicate first-degree relatives and black bars indicate second-degree relatives).

Table 1 Metabolic, immunological, and genetic markers in the children with another known autoimmune disease (AID) in addition to type 1 diabetes (T1D) and children with T1D only. Bold P values refer to significant differences.

	Children with additional AIDs (n=23)	Children with T1D only (n=1465)	Unadjusted P value	Adjusted P value ^a
Demographics				
Sex: male (%)	43.5	57.1	0.21	
Age at diagnosis (years), median (range)	9.5 (4.3–14.99)	8.2 (0.28–14.98)	0.06	
Number of children in the family, median (mean)	2 (2.61)	2 (2.40)	0.88	
Positive extended family history of T1D (%)	21.7	21.8	1.00	
Metabolic decompensation at diagnosis				
Plasma glucose (mmol/l), median (range)	18.6 (6.0–41.1)	24.5 (3.2–97.6)	0.004	<0.001
Ketoacidosis (%)	4.3	19.1	0.13	0.09
pH, median (range)	7.40 (7.27–7.51)	7.38 (6.80–7.54)	0.001	0.007
β-Hydroxybutyrate (mmol/l), median (range)	0.3 (0–6.0)	1.8 (0–20.1)	0.004	<0.001
Impaired consciousness (%)	0.0	5.1	0.62	1.00
Duration of symptoms (days), median (range)	21.5 (0–61)	9.0 (0–377)	0.03	0.65
Weight loss (kg), median (range)	1.1 (0–5.0)	1.4 (0–20.0)	0.50	0.09
Autoantibodies				
ICA (%)	78.3	93.4	0.01	0.02
ICA (JDFU), median (range)	40 (4–640)	40 (2.5–39 935)	0.97	1.00
IAA (%)	30.4	44.4	0.21	0.40
IAA (RU), median (range)	7.9 (4.7–22.3)	10.3 (2.9–309.3)	0.35	0.77
IA-2A (%)	60.9	76.0	0.14	0.10
IA-2A (RU), median (range)	74.5 (0.9–193.5)	101.8 (0.9–553.3)	0.39	0.68
GADA (%)	65.2	66.9	1.00	0.86
GADA (RU), median (range)	81.6 (6.5–419.6)	39.4 (5.4–812.4)	0.13	<0.001
Number of positive antibodies, median (mean)	2 (2.35)	3 (2.81)	0.14	0.10
Genetics				
DR3-DQ2/DR4-DQ8 (%)	13.0	22.0	0.33	0.33
DR3-DQ2/x ^b (%)	26.1	16.2	0.25	0.19
DR4-DQ8/y ^c (%)	47.8	46.6	1.00	0.93
x ^b /y ^c	13.0	15.2	1.00	0.73
DR4-DQ8 positive (%)	60.9	68.7	0.50	0.43
DR3-DQ2 positive (%)	39.1	38.1	1.00	0.86

^aThe results are adjusted for the difference in age at diagnosis.^bx≠DR4-DQ8.^cy≠DR3-DQ2.

families, i.e. the number of children in the family was higher ($P<0.05$) in most comparisons. The number of children in the family did not significantly affect the variables tested, however, and thus was not included in the analysis as a confounding factor.

Familial T1D of these children has been described earlier (29); 21.8% had a positive family history of T1D among the extended family. The frequency of familial T1D did not differ according to the positive or negative history of other AIDs in the index child (Table 1) or in the extended family (23.8 vs 21.0%, $P=0.25$).

Autoantibodies

After adjustment for the difference in age at diagnosis, the children with a known additional AID at the presentation of T1D were less often positive for ICAs and had higher levels of GADAs (Table 1). The children with relatives affected by AIDs had higher levels of ICAs and IA-2A than those with no family history of AIDs, whereas no differences were observed in the autoantibody profile in the children from autoimmune families (Table 2).

Higher levels of ICAs were observed in the children with rheumatoid diseases (44 vs 40 JDF units, $P=0.001$) or AIT among relatives (44 vs 40 JDF units, $P=0.04$). The children with rheumatoid diseases among relatives had, in addition, higher levels of IA-2A (109.7 vs 99.5 RU, $P=0.006$). The children with or without a positive family history of CD did not differ in terms of autoantibodies.

Genetics

HLA risk haplotypes or genotypes of the 23 children with another known AID at diagnosis did not differ from those of the subjects with T1D only (Table 1). HLA genetic profile was similar in the children with or without a positive family history of AIDs and those from autoimmune families (Table 2).

The children with relatives affected by AIT or rheumatoid diseases in the extended family did not differ from those without a family history of AIDs in terms of HLA genetics. The children with a positive family history of CD, however, carried more often the DR3-DQ2 haplotype (66.0 vs 38.9%, $P<0.001$),

Table 2 Metabolic, immunological, and genetic markers in index cases with first- and/or second-degree relatives affected by autoimmune diseases (AIDs, Group Ia) and in index cases from autoimmune families (greater than three AID diagnoses and/or greater than two different AIDs in the extended family, Group Ib) compared with the children with no family history of AIDs (Group II). Bold P values refer to significant differences.

	Group Ia: children with AIDs in the extended family (n=356)	Group Ib: children from autoimmune families (n=150)	Group II: children with no additional autoimmunity in the extended family (n=807)	Group Ia vs II: P value	Group Ib vs II: P value
Demographics					
Sex: male (%)	58.7	57.3	55.6	0.36	0.77
Age at diagnosis (years), median (range)	8.15 (0.70–14.99)	8.77 (0.95–14.99)	8.32 (0.69–14.98)	0.47	0.29
Age of father at diagnosis (years), median	40.5	41.3	39.7	0.23	0.005
Age of mother at diagnosis (years), mean	38.2	39.0	37.8	0.30	0.02
Number of children in the family, median (mean)	2 (2.42)	2 (2.73)	2 (2.32)	0.05	0.009
Metabolic decompensation at diagnosis					
Plasma glucose (mmol/l), median (range)	25.1 (3.2–64.6)	22.9 (3.2–63.7)	24.8 (4.5–92.7)	0.64	0.003
Ketoacidosis (%)	19.4	14.2	22.3	0.31	0.04
pH, median (range)	7.38 (6.88–7.52)	7.39 (6.98–7.54)	7.37 (6.87–7.52)	0.11	< 0.001
β-Hydroxybutyrate (mmol/l), median (range)	1.9 (0–15.1)	1.0 (0–17.4)	2.3 (0–13.2)	0.34	< 0.001
Impaired consciousness (%)	5.5	2.1	6.0	0.88	0.09
Duration of symptoms (days), median (range)	10.5 (0–377)	8.5 (0–143)	10 (0–314)	0.18	0.61
Weight loss (kg), median (range)	1.5 (0–20.0)	1.5 (0–12.0)	1.5 (0–17.0)	0.66	0.14
Autoantibodies					
ICA (%)	95.1	89.0	93.2	0.30	0.11
ICA (JDFU), median (range)	44 (2.5–640)	44 (3–640)	40 (3–39 935)	0.003	0.39
IAA (%)	46.1	38.6	43.5	0.46	0.32
IAA (RU), median (range)	10.3 (2.9–171.2)	11.1 (2.9–198.5)	10.0 (2.9–309.3)	0.59	0.29
IA-2A (%)	79.1	73.1	75.0	0.16	0.71
IA-2A (RU), median (range)	105.7 (0.9–553.3)	102.5 (0.9–553.3)	99.5 (0.9–501.0)	0.05	0.18
GADA (%)	68.1	70.3	66.7	0.68	0.44
GADA (RU), median (range)	41.3 (5.5–501.9)	45.2 (6.0–341.1)	41.0 (5.6–812.4)	0.82	0.77
Number of positive antibodies, median (mean)	3 (2.88)	3 (2.71)	3 (2.78)	0.30	0.49
Genetics					
DR3-DQ2/DR4-DQ8 (%)	21.7	20.9	21.3	0.93	1.00
DR3-DQ2/x ^a (%)	15.1	17.6	17.8	0.30	1.00
DR4-DQ8/y ^b (%)	46.9	50.7	44.9	0.59	0.23
x ^a /y ^b	16.3	10.8	15.9	0.96	0.14
DR4-DQ8 positive (%)	68.7	71.6	66.3	0.47	0.24
DR3-DQ2 positive (%)	36.9	38.5	38.9	0.55	1.00

^ax≠DR4-DQ8.

^by≠DR3-DQ2.

the DR3-DQ2/DR4-DQ8 genotype (35.8 vs 21.3%, $P=0.02$), and the DR3-DQ2/nonDR4-DQ8 genotype (30.2 vs 17.8%, $P=0.04$). The frequencies of nonDR3-DQ2/DR4-DQ8 (30.2 vs 44.9%, $P=0.05$) and nonDR3-DQ2/nonDR4-DQ8 (3.8 vs 15.9%, $P=0.03$) genotypes were reduced.

Metabolic decompensation at diagnosis

After adjustment for the age at diagnosis, the children with another AID already at the presentation of T1D had lower plasma glucose levels, higher pH and lower β-hydroxybutyrate levels (Table 1). Similarly, the children from autoimmune families had lower plasma glucose level and were less acidotic, whereas those with or without a positive family history of other AIDs did not differ (Table 2).

A positive family history of AIT or rheumatoid diseases did not reveal any differences in the markers of metabolic decompensation of the index child.

The children who had relatives with CD had symptoms for a longer duration than those without any family history of AIDs (15 vs 10 days, $P=0.02$).

Discussion

In this cross-sectional population-based study, we characterized the family history of AIDs in Finnish families with a diabetic child and looked for differences in diabetes phenotype and genotype depending on such a history. As the HLA-DR3-DQ2 haplotype is a risk haplotype for AIDs (20, 22, 25), especially for CD (11, 19, 21, 23, 24, 25), we expected it to be more prevalent among the children with a positive family history of AIDs. Such an increase in the frequency of the haplotype was not observed, in general, but the haplotype was strongly associated with a positive family history of CD. In addition, we postulated that a positive family history of AIDs would result in a stronger

humoral immune response to β -cell antigens. A broad activation of autoimmune reactions reflected by multiple different AIDs in the family could translate into a broad activation of immune reactions against T1D-related autoantigens in the index child. We did observe higher ICA and/or IA-2A titers in the children with a positive family history of AIDs, but no differences in the frequencies of autoantibodies were evident. In summary, although we found some differences in β -cell autoimmunity in the children with a positive family history of AIDs, the differences were modest. To further characterize the effects of a positive family history of AIDs, we grouped the index children into autoimmune families, in which autoimmunity was truly clustered, and accordingly, differences in pathogenetic mechanisms should be evident. The only significant difference observed, however, was the milder metabolic decompensation at diagnosis among the children from autoimmune families, which is probably due to the effect of familial diabetes and the earlier recognition of symptoms in such families (29). These findings do not consistently support the hypothesis of different pathogenetic mechanisms of diabetes operating in families with clustered autoimmunity.

Our dataset included only 23 children (1.5%) with another AID already known at T1D diagnosis. This is in keeping with previous studies in that additional autoimmunity is generally diagnosed after the clinical presentation of T1D (8, 9, 14). We did not screen for organ-specific autoantibodies such as tissue transglutaminase, 21-hydroxylase, and thyroid autoantibodies and were accordingly not able to diagnose subclinical conditions. This makes our prevalence of additional autoimmunity lower than that reported previously for children at diagnosis (8, 10). The children with multiple AIDs tended to be older at diagnosis, and they had somewhat milder metabolic decompensation. The duration of symptoms did not differ, however, after adjustment for age at diagnosis. These findings suggest a milder clinical manifestation of T1D in pediatric patients with a known additional AID. This seems to be not due to earlier diagnosis, but rather suggests a slower diabetic disease process. Accordingly, some differences in β -cell autoimmunity were also observed; these children had ICAs less frequently and had higher levels of GADAs after adjustment for age at diagnosis. This is in line with GADAs being associated with a general propensity to develop autoimmunity (14, 20, 22, 26). However, no inverse relationship between IA-2A and additional autoimmunity (20) or differences in genetics were observed possibly due to the small number of cases.

One-third of the index children had at least one extended family member affected by an AID other than T1D. In previous reports, this proportion has ranged from 20 to 70% (1, 2, 3, 8, 18, 20, 36). These studies, however, vary in terms of T1D duration, possible screening of family members for autoantibodies, and the categories of relatives included in the analysis. The index cases had

more often second-degree relatives than first-degree ones affected by AIDs (Fig. 2). This can be readily explained by older age and thus longer follow-up of second-degree relatives. As the total number of second-degree relatives is unknown, we are unable to report the actual prevalence of AIDs in this group. Families with a positive family history of AIDs had a higher number of children and the parents were older at the diagnosis of the index case, reflecting that a larger family size and a longer observation time increase the chances for AID diagnoses. Interestingly, parity has been identified as a risk factor for many AIDs (37), and this could contribute to the increased prevalence of AIDs in larger families.

In accordance with previous findings, mothers were more often affected by an AID other than T1D compared with fathers (16, 18, 38). Similarly, maternal grandparents and maternal relatives, in general, were affected by AIDs more often than paternal counterparts. This could reflect the increased genetic risk of autoimmunity in the relatives of mothers or a recollection bias in the form of mothers being more aware than fathers of the diseases diagnosed in their relatives. Interestingly, in the same dataset, such a difference was not observed for T1D; it was reported equally often in maternal (6.5%) and paternal (6.0%) second-degree relatives, but was more common in fathers (6.2%) than in mothers (3.2%) (29). This emphasizes the differential sex distributions and possible sex-dependent inheritance patterns in T1D and other AIDs. Despite these differences, based on the general clustering of autoimmunity, an increased prevalence of T1D could be expected in families with multiple AID diagnoses. However, the prevalence of familial T1D did not differ between the children from families with a positive or negative family history of other AIDs.

The asset of our study is that the subjects were derived from a nationwide register, which includes more than 90% of pediatric patients with T1D diagnosed in a country with the highest incidence of T1D globally. This high level of ascertainment and the large sample were made possible by relying on the information provided by the questionnaires on the extended family history of AIDs. The questionnaires were completed with the help of health care professionals, and the intention was to ensure the autoimmune origin of the reported diseases. Despite the efforts, this cannot be guaranteed, as the family members were not systematically contacted to confirm the diagnosis. If the questions about AIDs in the questionnaires were left unanswered, the family was counted as not having any family members affected by AIDs. Accordingly, our results can be expected to be conservative and to some extent underestimate the proportion of children with AIDs in their relatives. In addition, only clinically manifested autoimmunity was included in our questionnaire-based study, leaving subclinical conditions undetected. Consequently, the ascertainment of AIDs is the major limitation of our study as strict ascertainment was not possible in such a large dataset.

This study did not provide consistent support for the differential pathogenetic mechanisms of T1D in families with clustered autoimmunity; enrichment of HLA class II risk haplotypes was not observed, and only modest differences in autoantibody profile were apparent. Clustered autoimmunity in the extended family was, however, a fairly common phenomenon with one-third of the children having a first- or second-degree relative diagnosed with an AID. Reasons for this clustering still remain unclear and call for further studies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

A Parkkola analyzed the data, wrote the first version of the manuscript, and edited the manuscript; T Härkönen was in charge of the autoantibody laboratory, reviewed the manuscript, and contributed to the discussion; J Ilonen was responsible for the HLA genotyping, reviewed the manuscript, and contributed to the discussion; S J Ryhänen reviewed the manuscript and contributed to the discussion; and M Knip planned the study, contributed to the discussion, and reviewed the manuscript. The participants of the Finnish Pediatric Diabetes Register were involved in the planning of the study design and collection of data.

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