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TYPE 1 DIABETES: PATHOPHYSIOLOGY AND PREVENTION

Associations between deduced first islet specific autoantibody with sex, age at diagnosis and genetic risk factors in young children with type 1 diabetes

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Abstract

Objectives: We aimed to further characterize demography and genetic associations of type 1 diabetes "endotypes" defined by the first appearing islet specific autoantibodies.

Research Design and Methods: We analyzed 3277 children diagnosed before the age of 10 years from the Finnish Pediatric Diabetes Register. The most likely first autoantibody could be deduced in 1636 cases (49.9%) based on autoantibody combinations at diagnosis. Distribution of age, sex, HLA genotypes and allele frequencies of 18 single nucleotide polymorphisms (SNPs) in non-HLA risk genes were compared between the endotypes.

Results: Two major groups with either glutamic acid decarboxylase (GADA) or insulin autoantibodies (IAA) as the deduced first autoantibody showed significant differences in their demographic and genetic features. Boys and children diagnosed at young age had more often IAA-initiated autoimmunity whereas GADA-initiated autoimmunity was observed more frequently in girls and in subjects diagnosed at an older age. IAA as the first autoantibody was also most common in HLA genotype groups conferring high-disease risk while GADA first was seen more evenly and frequently in HLA groups associated with lower type 1 diabetes risk. The risk alleles in *IKZF4* and *ERBB3* genes were associated with GADA-initiated whereas those in *PTPN22*, *INS* and *PTPN2* genes were associated with IAA-initiated autoimmunity.

Conclusions: The results support the assumption that in around half of the young children the first autoantibody can be deduced based on islet autoantibody combinations at disease diagnosis. Strong differences in sex and age distributions as well as in genetic associations could be observed between GADA- and IAA-initiated autoimmunity.

KEYWORDS
autoantibodies, demography, genes, HLA antigens

A complete list of the investigators of the Finnish Pediatric Diabetes Register can be found in the Appendix section.

1 | INTRODUCTION

Type 1 diabetes is characterized by destruction of insulin producing beta cells in pancreatic islets due to an autoimmune response. This response is demonstrated by the appearance of specific autoantibodies and circulating T cells recognizing various protein antigens present in endocrine islet cells as well as by insulitis characterized by infiltration of mononuclear cells, especially CD8+ T cells into pancreatic islets.[1] The peak incidence of the disease is observed in childhood decreasing thereafter but it can appear at any age.[2] Despite the peak incidence in childhood the number of patients developing the disease in adulthood may still be higher because of longer time of case accumulation.[3] Differences between the disease contracted by children and adults are well known. The disease in children is usually developing more rapidly and also the panel of autoantibodies present is larger. Particularly insulin autoantibodies (IAA) are more common in children than in adults whereas antibodies to glutamic acid decarboxylase 65 (GADA) dominate in adult patients. HLA genotypes associated with the disease are also found in a higher proportion of patients diagnosed in childhood than in adulthood.[4]

There is, however, heterogeneity also within type 1 diabetes diagnosed in children. Although differences such as the higher frequency of IAA most conspicuously among the youngest children have been known for a long period,[5–7] the recent follow-up studies in children with genetic risk of type 1 diabetes have revealed two major disease forms, that is, endotypes which may differ in underlying biological mechanisms.[8] These two endotypes are characterized by the specificity of the first autoantibody reflecting the initiation of the autoimmune response, most commonly either IAA or GADA is detected first. As the first autoantibody, IAA appears usually earlier with a peak before 2 years of age while the peak of GADA as the first autoantibody peaks later during childhood, around the age of 4–5 years, followed by a relatively even appearance continuing throughout childhood.[9–11] These two endotypes have also different associations with specific HLA alleles and differences in their associations with non-HLA risk genes.[9–13] The two other of the best characterized autoantibodies, those recognizing insulinoma associated antigen 2 (IA-2A) or zinc transporter 8 (ZnT8A) are relatively rare as first appearing autoantibodies but all four major islet autoantibodies also develop as secondary autoantibodies predicting development of clinical type 1 diabetes and are all common at the diagnosis especially in young children.[14]

In the current study, we aimed at further exploring the heterogeneity of childhood type 1 diabetes by analyzing demographic and genetic features of endotypes characterized by various initial autoantibodies. The Finnish Pediatric Diabetes Register (FPDR) sample collection was utilized in the analysis.[15] Genetic analyses included comparison of major risk groups defined by various genotypes of HLA class II (DR/DQ) haplotypes as well as SNPs in non-HLA genes which have been found most strongly associated with T1D susceptibility in European populations and FPDR samples.[16,17] The two main groups of children with IAA- or GADA-initiated autoimmunity were the primary target of the analysis. Observations made in the follow-up program of the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) study allowed us to deduce the most likely first autoantibody reflecting the islet specific autoimmunity in a high proportion of children diagnosed with type 1 diabetes. This was based on the strong correlation of the first appearing autoantibody with certain combinations of islet specific autoantibodies detected at the diagnosis of the disease.[14] Our hypothesis is that the large number of children available at diagnosis as well as inclusion of all HLA genotypes of children with T1D might reveal new features of the major endotypes compared to follow-up studies with relatively small numbers of diagnosed children and HLA genotypes limited to those with high risk of the disease.

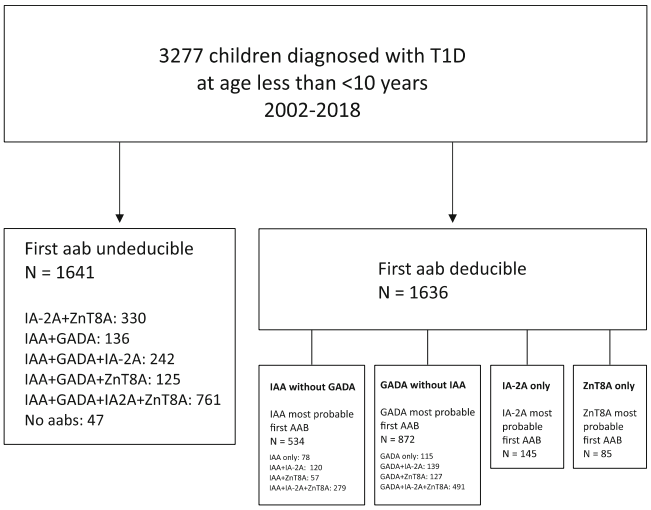

FIGURE 1 Description of various autoantibody combinations observed among young children diagnosed with T1D in the Finnish Pediatric Diabetes Register. If IAA without GADA or GADA without IAA was present at diagnosis these were deduced to be probably also first autoantibodies to appear like also IA-2A and ZnT8A when these were the only autoantibodies present

2 | METHODS

2.1 | Study population

The study protocol of the FPDR has been approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa. All of the 3277 children diagnosed with type 1 diabetes before the age of 10 years in Finnish hospitals with a pediatric unit and participating in the FPDR were included in the analysis. In 1636 of them (49.9%) the likely first appearing autoantibody could be deduced. Data on gender (except one case) and age at diagnosis was available for analysis. There was no differences between distribution of girls and boys or HLA risk groups between children whose likely first appearing autoantibody could be deduced and those where it was not possible but a significant difference in age distribution was detected median age being 6.4 years among those where first autoantibody could be deduced and 5.2 years in other children ($p < 0.0001$, Table S1).

2.2 | Autoantibody analysis and deduction of the first appearing autoantibody

Serum samples for measurement of autoantibodies against GAD65, insulin, the IA-2 antigen and zinc transporter 8 were collected within 2 weeks after the diagnosis of type 1 diagnosis. Radio-binding assays were used as described earlier.[15,18] The first autoantibody reflecting the initiation of islet specific autoimmunity was deduced according to the autoantibody patterns at diagnosis as described earlier in a follow-up analysis of the DIPP study where the identified first autoantibodies were compared with autoantibody patterns at diagnosis[14] (Figure 1). In short, when IAA but not GADA was present at diagnosis (independent of IA-2A and ZnT8A) IAA was in most cases (78%) the first autoantibody and GADA only in 6%. Respectively GADA without IAA at diagnosis was in 72% also the first autoantibody and IAA in 20%. IA-2A or ZnT8A alone at diagnosis was also deduced to be the initial autoantibody present. Only children diagnosed before the age of 10 years were included into these analyses as an increasing frequency of inverse seroconversion for IAA is observed by rising age.[14]

2.3 | Analysis of HLA DR/DQ genotypes

HLA DR-DQ genotypes were defined using a step-wise procedure starting from DQB1 genotyping and continuing with DQA1 and DRB1 typing when relevant for distinguishing haplotypes associated with susceptibility to or protection against type 1 diabetes.[19] HLA genotypes were divided into risk groups based on observed risk levels when in 2991 Finnish trio families when children diagnosed with type 1 diabetes were compared with affected family based artificial controls formed from non-transmitted haplotypes in trio families composed of the affected child and two healthy parents.[19]

Children were classified into six different risk groups and the highest risk was associated with heterozygous combinations of two different major risk haplotypes: (DR3)-DQA1*05-DQB1*02 and DRB1*04:01/2/4/5-DQA1*03-DQB1*03:02 where the Odds Ratio (OR) was 13.2. The moderate risk group (OR 7.2) consisted of homozygotes for either one of these two haplotypes and of genotypes where DRB1*04:01/2/5-DQA1*03-DQB1*03:02 was associated with any of the neutral haplotypes: (DR1/10)-DQB1*05:01, (DR4)-DQA1*03-DQB1*03:01, (DR7)-DQA1*0201-DQB1*02, (DR8)-DQB1*04, (DR9)-DQA1*03-DQB1*03:03, (DR13)-DQB1*06:04/9, (DR16)-DQB1*05:02. The slightly increased risk group (OR 1.9) combined either (DR3)-DQA1*05-DQB1*02 or DRB1*04:04-DQA1*03-DQB1*03:02 haplotype with a neutral haplotype or DRB1*04:01/2/5-DQA1*03-DQB1*03:02 haplotype with a weak protective haplotype: DRB1*0403-DQA1*03-DQB1*03:02, (DR11/12/13)-DQA1*05-DQB1*0:301 or (DR13)-DQB1*06:03. The neutral risk group (OR = 0.38) included combinations of various neutral haplotypes as well as combinations of DRB1*04:01/2/5-DQA1*03-DQB1*03:02 with strong protection associated haplotypes: (DR15)-DQB1*06:01, (DR15)-DQB1*06:02, (DR7)–DQA1*02:01–DQB1*03:03 and (DR14)-DQB1*05:03. The original groups associated with slightly (OR = 0.10) and strongly decreased risk (OR = 0.02) were combined into one decreased risk group (OR = 0.04) because of the small numbers of these genotypes among children with T1D.

2.4 | Analysis of non-HLAgene polymorphisms

A panel of 19 SNPs in genes outside the HLA region confirmed to be associated with type 1 diabetes susceptibility was analyzed.[16,17] These included rs12708716 (*CLEC16A*), rs12722495 (*IL2R*), rs1701704 (*IKZF4*), rs17388568 (*ADAD1*), rs1990760 (*IFIH1*), rs2104286 (*IL2R*), rs2292239 (*ERBB3*), rs2476601 (*PTPN22*), rs2816316 (*RGS1*), rs3087243 (*CTLA4*), rs3184504 (*SH2B3*), rs3757247 (*BACH2*), rs3825932 (*CTSH*), rs45450798 (*PTPN2*), rs601338 (*FUT2*), rs689 (*INS*), rs6920220 (*TNFAIP3*), rs7574865 (*STAT4*) and rs9976767 (*UBASH3A*). A part of the study population was earlier genotyped in our study on the association of these non-HLA genotypes with type 1 diabetes in trio families of children from the FPDR[17] using the Sequenom (San Diego, CA, USA) platform, (Genome Center of Eastern Finland, University of Eastern Finland, Kuopio). The expanded analysis for this study was carried out using Open Array® technology (Thermo Fisher Scientific, Waltham, MA, USA) at Turku Bioscience. From 1613 (rs3087243) to 1634 (rs17388568 and rs2476601, respectively) children were successfully genotyped for the selected SNPs.

2.5 | Statistical analyses

The statistical analysis was started using univariate analysis to explore relationships between explanatory variables (sex, age, risk group and SNP) and the response variable, that is, the deduced first autoantibody group (Tables 1–3). Relationships between age and AAB group were examined with Kruskal–Wallis test and using Dwass-Steel method in pairwise multiple comparison. The Chi-square test was used for categorical variables. The data were presented as median with quartiles for age and counts with percentage for categorical variables. p Values were corrected with Benjamin and Hochberg FDR correction for multiple testing in SNP analysis. The level of significance was set at a corrected p value <0.05.

The deduced first autoantibody group (IAA vs. GADA) were analyzed also using logistic regression. Child's sex, age, risk group and SNP variables (rs1990760, rs2292239, rs2476601, rs45450798, rs689 and rs1701704) were included in the model as explanatory variables. The variables rs1701704 and rs2292239 were analyzed in different models due to their high correlation (Cramer's $V = 0.88$). The interactions were not statistically significant and were, therefore, removed from the final models. Univariate analysis and logistic models were also made separately for different age groups to examine the effect of age. The level of significance was set at p value <0.05. The analyses were carried out with the SAS software, version 9.4 for Windows (SAS Institute Inc., Cary, NC USA).

TABLE 1 Proportion of various deduced first autoantibodies in children diagnosed with type 1 diabetes before the age of 10 years

		Deduced first autoantibody				
	Total	GADA	IAA	IA-2A	ZnT8A	*p* Value
N	1636	872 (53.3)	534 (32.6)	145 (8.9)	85 (5.2)	
Sex						
Girls	761 (46.5)	497 (65.3)	181 (23.8)	49 (6.4)	34 (4.5)	
Boys	874 (53.5)	375 (42.9)	352 (40.3)	96 (11)	51 (5.8)	<0.0001
Age (years)	6.4 (4.2–8.2)	7.2 (5.4–8.7)	4.2 (2.6–6.5)	6.6 (4.7–8.3)	6.5 (5.1–8.3)	<0.0001
HLA risk group						
Decreased risk	40 (2.4)	26 (65)	7 (17.5)	2 (5)	5 (12.5)	
Neutral	231 (14.1)	136 (58.9)	55 (23.8)	22 (9.5)	18 (7.8)	
Slightly increased	384 (23.5)	238 (62)	105 (27.3)	23 (6)	18 (4.7)	
Moderately increased	630 (38.5)	290 (46)	241 (38.3)	72 (11.4)	27 (4.3)	
High risk	351 (21.5)	182 (51.9)	126 (35.9)	26 (7.4)	17 (4.8)	<0.0001

Note: Continuous data (age) are expressed as median (quartiles Q1–Q3) and the differences between groups were assessed using Kruskal–Wallis test. Categorical data are expressed as number (%) and were assessed using the Chi–square test.

TABLE 2 Genotype frequencies of type 1 diabetes associated non-HLA SNPs compared between children diagnosed with type 1 diabetes who had either GADA or IAA as the deduced first autoantibody

SNP and gene	SNP genotypes	Total *N*	GADA *N* (%)	IAA *N* (%)	*p* Value	Corrected *p* value	Dominant *p value*	Recessive *p* value
Risk allele associated with GADA as the deduced first autoantibody								
rs1701704	AA	542	311 (36.0)	231 (43.8)				
IKZF4	**A**C	671	427 (49.4)	244 (46.2)				
	CC	179	126 (14.6)	53 (10.0)	**0.004**	**0.031**	**0.004**	**0.014**
rs1990760	CC	213	125 (14.5)	88 (16.8)				
IFIH1	C**T**	693	420 (48.6)	273 (52.0)				
	TT	484	320 (37.0)	164 (31.2)	0.081	0.23	0.25	**0.029**
rs2292239	**AA**	163	110 (12.7)	53 (10.0)				
ERBB3	**A**C	659	427 (49.4)	232 (43.9)				
	CC	572	328 (37.9)	244 (46.1)	**0.0085**	**0.043**	**0.0025**	0.13
Risk allele associated with IAA as the deduced first autoantibody								
rs2476601	**AA**	73	42 (4.8)	31 (5.8)				
PTPN22	**A**G	449	259 (29.7)	190 (35.7)				
	GG	882	571 (65.5)	311 (58.5)	**0.031**	0.12	**0.0083**	0.41
rs45450798	**CC**	49	24 (2.8)	25 (4.7)				
PTPN2	**C**G	388	221 (25.4)	167 (31.4)				
	GG	964	624 (71.8)	340 (63.9)	**0.0046**	**0.031**	**0.002**	0.055
rs689	**AA**	1086	638 (73.3)	448 (84.1)				
INS	**A**T	290	209 (24.0)	81 (15.2)				
	TT	27	23 (2.6)	4 (0.8)	**<0.0001***	**0.0002**	**<0.0001***	**0.015***

Note: Only SNPs where significant differences between these groups were found are presented. Data are expressed as number (%) of each SNP genotype and were assessed using the Chi–square test. *p* Values calculated for differences in distribution of three genotypes as well as *p* values corrected with Benjamin and Hochberg FDR method. Also uncorrected *p* values for dominant and recessive model are presented. SNP risk alleles and significant *p* values marked in bold.

*Fisher's exact test was used because limited number of subjects.

3 | RESULTS

3.1 | Age, sex and HLA-DR/DQ associated risk groups in relation to deduced first autoantibodies

The median age of the 1636 children whose first autoantibody could be deduced was 6.40 years. There were 761 (46.5%) girls and 874 boys (53.5%), among the 1635 children with available information. No significant difference in the age distribution between girls (median 6.5, interquartile range 4.4–8.2) and boys (median 6.2, interquartile range 4.0–8.1) was detected (Wilcoxon test). Table 1 presents distribution of sex, age and HLA-DR/DQ risk groups in children with various deduced first antibodies. Highly significant differences were observed for each parameter. Boys developed more often IAA, IA-2A, and ZnT8A as the deduced first autoantibody while GADA was most often found in girls. Median age at diagnosis in children with IAA as the deduced first autoantibody was youngest (4.2 years) and those with GADA oldest (7.2 years). The median age at diagnosis of the smaller groups with either IA-2A (6.6 years) or ZnT8A (6.5 years) as the first autoantibody was quite close to those with GADA as the first autoantibody.

Significant differences were detected when the distribution of children with various deduced first autoantibodies was compared between groups based on disease risk conferred by various HLA class II genotypes. Children with IAA as the first autoantibody were more often observed in the high and moderately increased HLA risk groups whereas the highest proportion of children with GADA as the first autoantibody was seen among children with genotypes conferring slightly increased, neutral and decreased disease risk. The proportion of children with IA-2A as the deduced first autoantibody was highest in the group with moderately increased risk similarly to the children with IAA as the deduced first autoantibody and they differed significantly from those with GADA as the deduced first autoantibody while children with ZnT8A as the first autoantibody differed from the group of children with IAA as the first autoantibody resembling more those with GADA as the deduced first autoantibody. The proportion of children with high- and moderate-risk genotypes was 68.7% when IAA was the deduced first autoantibody compared to 54.1% in those with GADA as their deduced first autoantibody.

In the group where GADA was deduced to be the first autoantibody (DR3)-DQA1*05-DQB1*02, DRB1*04:04-DQA1*03-DQB1*03:02 and (DR1/10)-DQB1*05:01 haplotypes were significantly more frequent whereas DRB1*04:01-DQA1*03-DQB1*03:02 and (DR8)-DQB1*04 were increased among those with IAA as the first autoantibody (Table S2). No significant differences between these most common haplotypes were observed when the group where the first autoantibody was undeducible was compared with the group where the first autoantibody could be deduced indicating that the distribution of first autoantibodies was probably quite similar in both groups. The frequencies of (DR3)-DQA1*05-DQB1*02 and DRB1*04:01-DQA1*03-DQB1*03:02 were similar to the GADA-first group in children positive for ZnT8A only and similar to the IAA-first group in those with IA-2A only. Because of the small size of these groups only the difference in (DR3)-DQA1*05-DQB1*02 remained significant after correction ($p = 0.04$).

3.2 | Risk alleles in non-HLA risk genes and deduced first autoantibodies

Differences in the frequency of alleles of SNPs in non-HLA risk genes were also observed between children with various deduced first autoantibodies. Table 2 shows allele frequencies of SNPs which showed significant differences between children with either IAA or GADA as the deduced first autoantibody to appear. *p* Values were calculated for differences between all three genotypes as well as for the dominant or recessive model of the risk allele.

The risk allele for type 1 diabetes was found to be associated with GADA as the first autoantibody in the case of rs1701704 and rs2292239 in the strongly linked *IKZF4* and *ERBB3* genes, respectively. A marginal association with GADA as the first autoantibody was also seen for the risk allele rs1990760 in the *IFIH1* gene although this association was no more significant after correction for multiple testing. IAA was instead more often observed as the deduced first autoantibody among the carriers of the type 1 diabetes risk alleles of rs2476601 in *PTPN22*, rs45450798 in *PTPN2* and rs689 in *INS* genes.

TABLE 3 Odd ratios for the IAA versus GADA from logistic regression

	OR (95% CI)	Overall *p* value
Risk group Decreased risk vs. Neutral	0.81 (0.29, 2.27)	
Risk group Slightly increased vs. Neutral	1.10 (0.70, 1.75)	
Risk group Moderately increased vs. Neutral	2.09 (1.36, 3.21)	
Risk group high risk vs. neutral	1.57 (0.98, 2.51)	0.0003
Sex female vs. male	0.31 (0.23, 0.40)	<0.0001
Age	0.63 (0.59, 0.67)	<0.0001
rs1990760 CC vs. TT	1.44 (0.96, 2.15)	
rs1990760 CT vs. TT	1.40 (1.05, 1.88)	0.053
rs2292239 AA vs. CC	0.71 (0.45, 1.11)	
rs2292239 AC vs. CC	0.68 (0.52, 0.90)	0.022
rs2476601 AA vs. GG	1.14 (0.64, 2.03)	
rs2476601 AG vs. GG	1.67 (1.26, 2.23)	0.0021
rs45450798 CC vs. GG	2.00 (0.95, 4.19)	
rs45450798 CG vs. GG	1.30 (0.97, 1.73)	0.057
rs689 AA vs. TT	5.49 (1.43, 21.1)	
rs689 AT vs. TT	2.46 (0.63, 9.69)	<0.0001

Note: The model includes sex, age, HLA risk groups and SNPs rs1990760, rs2292239, rs2476601, rs45450798 and rs689 as explanatory factors. OR >1 indicates that deduced first autoantibody is more likely IAA than GADA compared to the reference category and vice versa for OR <1
(OR = adjusted odds ratio, CI = confidence interval).

TABLE 4 Comparison of allele frequencies of SNPs in T1D associated non-HLA genes in children diagnosed with T1D who had either GADA or IAA as their deduced first autoantibody and frequencies detected in the Finnish control population

		Deduced first autoantibody in children with T1D								
		Controls	GADA				IAA			
SNP and gene	Allele	*N* (%)	*N* (%)	OR (95% CI)	*p* Value	Corrected *p* value	*N* (%)	OR (95% CI)	*p* Value	Corrected *p* value
rs1701704	A	2298 (68.8)	1049 (60.7)	1.43 (1.27–1.62)			706 (66.9)	1.1 (0.95–1.27)		
IKZF4	**C**	1040 (31.2)	679 (39.3)		**<0.0001**	**<0.0001**	350 (33.1)		0.23	0.27
rs1990760	C	1393 (41.4)	670 (38.7)	1.12 (0.99–1.26)			449 (42.8)	0.95 (0.82–1.09)		
IFIH1	**T**	1971 (58.6)	1060 (61.3)		0.065	0.078	601 (57.2)		0.44	0.44
rs2292239	**A**	888 (29.8)	647 (37.4)	1.41 (1.24–1.6)			338 (31.9)	1.11 (0.95–1.29)		
ERBB3	C	2096 (70.2)	1083 (62.6)		**<0.0001**	**<0.0001**	720 (68.1)		0.18	0.27
rs2476601	**A**	484 (14.0)	343 (19.7)	1.51 (1.3–1.76)			252 (23.7)	1.91 (1.61–2.27)		
PTPN22	G	2980 (86.0)	1401 (80.3)		**<0.0001**	**<0.0001**	812 (76.3)		**<0.0001**	**<0.0001**
rs45450798	**C**	540 (16.1)	269 (15.5)	0.96 (0.82–1.12)			217 (20.4)	1.34 (1.12–1.59)		
PTPN2	G	2820 (83.9)	1469 (84.5)		0.58	0.58	847 (79.6)		**0.0011**	**0.0022**
rs689	**A**	2613 (77.6)	1485 (85.3)	1.68 (1.44–1.97)			977 (91.7)	3.17 (2.52–4.00)		
INS	T	755 (22.4)	255 (14.7)		**<0.0001**	**<0.0001**	89 (8.3)		**<0.0001**	**<0.0001**

Note: The statistical difference is calculated using Chi-square test. OR, odds ratio; 95% CI, 95% confidence interval. SNP risk alleles and significant *p* values marked in bold.

There were no significant differences after FDR correction between groups where the first autoantibody could be deduced or was undeducible (data not shown).

The associations of sex, age, HLA risk groups and SNPs with the deduced major first autoantibody groups, IAA versus GADA, were also analyzed using logistic regression. In this model sex, age, HLA risk groups and SNPs from Table 2 were explanatory variables although rs1701704 was left out as it was too strongly correlated with rs2292239 which was included in the final model. We first excluded that there were no significant interactions between the explanatory factors. The results shown in Table 3 demonstrated that all of them were differing between deduced IAA-first and GADA-first groups although the difference in the case of rs1990760 and rs45450798 remained at the borderline level of significance.

The frequency of risk alleles associated with GADA as the deduced first autoantibody (rs1701704 C in *IKZF4* and rs2292239 in *ERBB3)* was also higher compared to the frequency seen in the control population of our earlier study (constructed from family haplotypes not inherited to children with type 1 diabetes),[17] but their frequency in children with IAA-initiated autoimmunity did not differ from control values (Table 4). Similarly, in the case of *PTPN2* where the rs45450798 risk allele was associated with IAA as the first autoantibody the frequency of the risk allele was higher in children with IAA as the first autoantibody than in the control population but did not differ between children with GADA as the first autoantibody and the control population. However, in two genes, *INS* and *PTPN22*, where IAA as the deduced first autoantibody was significantly associated with the risk allele also children with GADA as the deduced first autoantibody had a strongly increased frequency of the risk alleles rs689 and rs2476601 SNPs when compared to controls.

4 | DISCUSSION

The analyses of demographic and genetic parameters of young Finnish children who at the diagnosis of type 1 diabetes showed islet autoantibody patterns implying either GADA or IAA as the first autoantibody revealed some new features of these major endotypes. The high proportion of IAA as the first deduced autoantibody in the youngest quartile and its rapid decrease thereafter as well as the concomitant increase of GADA-initiated autoimmunity is in accordance with the results obtained from follow-up studies starting from birth.[9–11] In this study we now detected a higher proportion of boys in those with IAA-initiated and of girls in GADA-initiated autoimmunity which parallels the finding of the TEDDY study that boys have higher probability for developing IAA than girls.[11] One has to note that the children included in this study have all developed type 1 diabetes and it is possible that the difference between boys and girls is actually in the probability to progress to clinical disease among those with IAA or GADA as their first autoantibody. It is well known that the incidence of type 1 diabetes between genders starts to differ more conspicuously in puberty when the incidence decreases rapidly in girls compared to boys[20,21] but this phenomenon was not yet seen in this study cohort where all children were younger than 10 years at diagnosis. In an earlier study of pediatric type 1 diabetes in Finland the difference between boys and girls began to appear just after age of 10 years but interestingly the incidence curve also suggests higher incidence in boys up to the age of 4 years after which the curve is very even until the age of 10 years.[22] One could also speculate that the increase in incidence earlier seen particularly in the youngest age group[22] below the age of 5 is related to the increase of the disease endotype characterized by IAA as the first autoantibody.

Strong differences were observed when children with various deduced first autoantibodies were compared for the genetic disease risk defined by HLA class II genotypes. The highest proportion of children with IAA as their first autoantibody was found in the moderately increased risk group followed tightly by the high-risk group decreasing thereafter concomitantly with the degree of HLA related risk. The moderately increased risk group consists of children homozygous for either DR4-DQ8 or DR3-DQ2 and a large group of children with a combination of the DRB1*04:01 positive (very rarely DRB1*04:02 or DRB1*04:05) DR4-DQ8 haplotype with "neutral" haplotypes fitting well with a high proportion of IAA as the first autoantibody. The proportion of children with GADA as the deduced first autoantibody was in contrast highest among those carrying HLA genotypes conferring slightly increased, decreased and neutral disease risk. In the highest risk group GADA-first endotype associated (DR3)-DQA1*05-DQB1*02 is always present and among DR4-DQ8 haplotypes in addition to DRB1*04:01-DQA1*03-DQB1*03:02 haplotype also the DRB1*04:04-DQA1*03-DQB1*03:02 haplotype, which in addition to (DR3)-DQA1*05-DQB1*02 was found to be associated more often with the GADA-first than the IAA-first endotype in our recent analysis of the DIPP study.[13]

The slightly increased risk group is defined as either DR3-DQ2 or DRB1*04:04 positive DR4-DQ8 combined with a "neutral" haplotype which is well in accordance with the association of an autoimmunity type where GADA is the first autoantibody. GADA-initiated autoimmunity was also dominating the neutral risk group comprising a mix of various combinations of neutral haplotypes and combinations of risk associated and protective haplotypes. Similarly, GADA dominated groups associated with decreased disease risk consisting mainly of combinations of neutral and protective haplotypes. Most neutral haplotypes were also observed to associate with GADA-initiated autoimmunity in our earlier study.[13] The clearly higher proportion of children with IAA as the first autoantibody in the two highest genetic risk groups, 68.7% compared to 54.1% in the GADA first group, implies also that a higher proportion of newborn children at particular risk for the endotype with IAA as the first autoantibody have been eligible for genetic follow-up in the DIPP study and criteria used in our HLA based screening more efficiently selected these children.

The distribution of children with IA-2A as the likely first autoantibody resembled children with IAA-initiated autoimmunity and those with ZnT8A children with GADA-initiated autoimmunity. These differences probably also reflect effects of specific class II HLA haplotypes. The associations of the DR4-DQ8 haplotype with IAA and the DR3-DQ2 haplotype with GADA are well known[23–26] and follow-up studies from birth confirmed these associations especially in the case of the first emerging autoantibody.[9–11] More precisely we recently demonstrated the IAA association to be seen particularly in those carrying the DRB1*04:01 allele in the DR4-DQ8 haplotype whereas the other common DR4 allele in this haplotype in the Northern European populations, DRB1*04:04, was actually more associated with GADA-initiated autoimmunity.[13] The difference was clear both in the DIPP follow-up study and in the deduced first autoantibody cohort in the largely overlapping FPDR series as analyzed here. A strong association of IA-2A as the only autoantibody with DRB1*04:01 was also implicated in our earlier study.[27]

The comparison of allele distribution in a panel of known type 1 diabetes associated SNPs in the non-HLA regions confirmed our earlier findings in both the DIPP and FPDR cohorts that the type 1 diabetes risk allele in the *INS* gene is associated with IAA-initiated and the risk allele in the *IKZF4-ERBB3* region with the GADA-initiated endotype of type 1 diabetes.[14,28] A weak association of *IFIH1* gene risk allele with GADA as the first autoantibody was also detected but this did not sustain statistical correction and remains waiting for confirmation in other studies. The current study also observed that both the *PTPN22* and *PTPN2* risk alleles are associated with the IAA-initiated disease process. The *INS* gene polymorphism associated with type 1 diabetes decreases thymic expression of insulin and thus impairs deletion of autoreactive T cells recognizing (prepro)insulin epitopes by their T-cell receptor and these cells remain in the body with the potential to launch insulin-specific autoimmunity in response to still poorly defined triggers.[29,30] No explanations for the mechanisms of endotype associations have so far been presented for endotype specificity of other non-HLA genes.

The specific association of type 1 diabetes risk associated *INS* gene polymorphism with the IAA-initiated islet autoimmunity has also been demonstrated in the TEDDY study when comparing those who first seroconverted to either IAA or GADA by the age of 6 years but no significant difference between children with these first autoantibodies and *PTPN22*, *PTPN2* or *ERBB3* was detected.[12] Instead, a significant difference was found in the case of *BACH2* which increased the risk of developing GADA as the first autoantibody and *CTLA4* which increased the risk of IAA as the first autoantibody compared to GADA.[12] In our study, we did not detect any significant difference in the distribution of either *BACH2* or *CTLA4* SNPs between diabetic children with IAA or GADA as deduced first autoantibodies. There are of course differences between this diabetes register based study and the TEDDY follow-up study where only a few high-risk genotypes representing about 50% of children with future type 1 diabetes were eligible for follow-up[31] whereas the present study is based on an unselected group covering most children diagnosed with type 1 diabetes during a relatively long-time period in the Finnish population. Only a part of TEDDY children with IAA or GADA as their first autoantibody will develop multiple autoantibodies and eventually type 1 diabetes. These differences may affect the discrepancies observed like also population-specific differences in frequency of, for example, the relatively rare *PTPN22* risk allele A which is common in Finland compared to most other European derived and especially non-European populations.[32] In our early analysis of first autoantibody association of *PTPN22* in the DIPP study it was strongly associated with IAA as the first autoantibody[33] and although the association did not reach significance in a subsequent study[28] the most recent analysis in the DIPP study suggests again a strong association (Laine et al. manuscript in preparation).

For most non-HLA genes where either the presence of GADA or IAA as the first autoantibody was associated with risk alleles the other major group of children had frequency of the risk allele not different

from that found in the Finnish background population. However, in the case of *PTPN22* and *INS* genes also children without a preferentially associated first autoantibody had an increased frequency of the risk allele compared to the control population. This may indicate that there are multiple mechanisms mediating the effects of these gene polymorphisms on disease susceptibility and only part of these are endotype specific. It is well known that the *PTPN22* polymorphism is associated with multiple autoimmune diseases in addition to type 1 diabetes. It affects lymphocyte development and activation, innate immunity, establishment of tolerance and immune regulation being likely involved in multiple stages of disease development.[34] The *INS* gene polymorphism instead is specific for type 1 diabetes and the effect is apparently limited to thymic selection. The *INS* and *PTPN22* polymorphisms are among the non-HLA genes those with the strongest effect on the risk of type 1 diabetes, and it may also be that associations of the risk alleles with the GADA-initiated autoimmunity are just due to the inaccuracy of our deduction method of the first specific autoantibody initiating the autoimmune response. Because IAA as the first autoantibody is most prone for reverse seroconversion a proportion of children classified to the group of GADA-initiated autoimmunity based on autoantibodies present at diagnosis may actually have had IAA as their first autoantibody. In our earlier follow-up study we found that 25% of children with IAA as the first autoantibody were negative for IAA at diagnosis.[14]

In conclusion, the current study supports the usefulness of our approach where the autoantibody panel present at diagnosis was used to deduce the first detectable autoantibody in young children with type 1 diabetes. We were able to observe significant differences between these endotypes in sex and age at diagnosis as well as in HLA risk groups and associations with SNPs in several non-HLA gene regions. A limitation of the study is of course that a large group of children with type 1 diabetes could not be included since the presence of both IAA and GADA at diagnosis prevented the deduction whether IAA or GADA was the likely first autoantibody. However, the size of the included group was large enough to detect several differences between the endotypes with either GADA and IAA as the first autoantibody.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

AUTHOR CONTRIBUTIONS

Jorma Ilonen and Johanna Lempainen planned the study, Jorma Ilonen analyzed the data and wrote the manuscript. Antti-Pekka Laine performed the SNP analyses. Minna Kiviniemi and Jorma Ilonen were responsible for HLA genotyping, Taina Härkönen and Mikael Knip for autoantibody analyses. All authors critically reviewed and edited the manuscript and approved the final manuscript.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The Ethics Committee of the Hospital District of Helsinki and Uusimaa has approved the study protocols. Study participants (aged ≥18) or their guardians gave their written informed consent. Participants aged 10–17 gave their written informed assent.

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REFERENCES

1. Clark M, Kroger CJ, Tisch RM. Type 1 diabetes: a chronic anti-self-inflammatory response. *Front Immunol*. 2017;8:1898.
2. Rawshani A, Landin-Olsson M, Svensson AM, et al. The incidence of diabetes among 0-34 year olds in Sweden: new data and better methods. *Diabetologia*. 2014;57(7):1375-1381.
3. Thomas NJ, Jones SE, Weedon MN, Shields BM, Oram RA, Hattersley AT. Frequency and phenotype of type 1 diabetes in the first six decades of life: a cross-sectional, genetically stratified survival analysis from UKbiobank. *Lancet Diabetes Endocrinol*. 2018;6(2):122-129.
4. Buzzetti R, Zampetti S, Maddaloni E. Adult-onset autoimmune diabetes: current knowledge and implications for management. *Nat Rev Endocrinol*. 2017;13(11):674-686.
5. Arslanian SA, Becker DJ, Rabin B, et al. Correlates of insulin antibodies in newly diagnosed children with insulin-dependent diabetes before insulin therapy. *Diabetes*. 1985;34(9):926-930.
6. Bingley PJ, Bonifacio E, Williams AJ, Genovese S, Bottazzo GF, Gale EA. Prediction of IDDM in the general population: strategies based on combinations of autoantibody markers. *Diabetes*. 1997;46(11):1701-1710.
7. Komulainen J, Kulmala P, Savola K, et al. Clinical, autoimmune, and genetic characteristics of very young children with type 1 diabetes. Childhood diabetes in Finland (DiMe) study group. *Diabetes Care*. 1999;22(12):1950-1955.
8. Battaglia M, Ahmed S, Anderson MS, et al. Introducing the endotype concept to address the challenge of disease heterogeneity in type 1 diabetes. *Diabetes Care*. 2020;43(1):5-12.
9. Ilonen J, Hammais A, Laine AP, et al. Patterns of β-cell autoantibody appearance and genetic associations during the first years of life. *Diabetes*. 2013;62(10):3636-3640.
10. Giannopoulou EZ, Winkler C, Chmiel R, et al. Islet autoantibody phenotypes and incidence in children at increased risk for type 1 diabetes. *Diabetologia*. 2015;58(10):2317-2323.
11. Krischer JP, Lynch KF, Schatz DA, et al. The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. *Diabetologia*. 2015;58(5):980-987.
12. Krischer JP, Lynch KF, Lernmark Å, et al. Genetic and environmental interactions modify the risk of diabetes-related autoimmunity by

6 years of age: the TEDDY study. *Diabetes Care*. 2017;40(9):1194-1202.
13. Mikk ML, Pfeiffer S, Kiviniemi M, et al. HLA-DR-DQ haplotypes and specificity of the initial autoantibody in islet specific autoimmunity. *Pediatr Diabetes*. 2020;21(7):1218-1226.
14. Ilonen J, Lempainen J, Hammais A, et al. Primary islet autoantibody at initial seroconversion and autoantibodies at diagnosis of type 1 diabetes as markers of disease heterogeneity. *Pediatr Diabetes*. 2018;19(2):284-292.
15. Parkkola A, Härkönen T, Ryhänen SJ, Ilonen J, Knip M. Finnish Paediatric diabetes register. Extended family history of autoimmune diseases and phenotype and genotype of children with newly diagnosed type 1 diabetes. *Eur J Endocrinol*. 2013;169(2):171-178.
16. Pociot F, Lernmark Å. Genetic risk factors for type 1 diabetes. *Lancet*. 2016;387(10035):2331-2339.
17. Laine AP, Knip M, Ilonen J. Finnish Paediatric diabetes register. Transmission disequilibrium analysis of 31 type 1 diabetes susceptibility loci in Finnish families. *Tissue Antigens*. 2013;82(1):35-42.
18. Juusola M, Parkkola A, Härkönen T, et al. Positivity for zinc transporter 8 autoantibodies at diagnosis is subsequently associated with reduced β-cell function and higher exogenous insulin requirement in children and adolescents with type 1 diabetes. *Diabetes Care*. 2016;39(1):118-121.
19. Ilonen J, Kiviniemi M, Lempainen J, et al. Genetic susceptibility to type 1 diabetes in childhood—estimation of HLA class II associated disease risk and class II effect in various phases of islet autoimmunity. *Pediatr Diabetes*. 2016;17(Suppl 22):8-16.
20. Dahlquist GG, Nyström L, Patterson CC. Swedish childhood diabetes study group, diabetes incidence in Sweden study group. Incidence of type 1 diabetes in Sweden among individuals aged 0-34 years, 1983-2007: an analysis of time trends. *Diabetes Care*. 2011;34(8):1754-1759.
21. Weets I, De Leeuw IH, Du Caju MV, et al. The incidence of type 1 diabetes in the age group 0-39 years has not increased in Antwerp (Belgium) between 1989 and 2000: evidence for earlier disease manifestation. *Diabetes Care*. 2002;25(5):840-846.
22. Harjutsalo V, Sjöberg L, Tuomilehto J. Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study. *Lancet*. 2008;371(9626):1777-1782.
23. Ziegler R, Alper CA, Awdeh ZL, et al. Specific association of HLA-DR4 with increased prevalence and level of insulin autoantibodies in first-degree relatives of patients with type I diabetes. *Diabetes*. 1991;40(6):709-714.
24. Vandewalle CL, Decraene T, Schuit FC, De Leeuw IH, Pipeleers DG, Gorus FK. Insulin autoantibodies and high titre islet cell antibodies are preferentially associated with the HLA DQA1*0301-DQB1*0302 haplotype at clinical type 1 (insulin-dependent) diabetes mellitus before age 10 years, but not at onset between age 10 and 40 years. The Belgian Diabetes Registry. *Diabetologia*. 1993;36(11):1155-1162.
25. Vandewalle CL, Falorni A, Lernmark A, et al. Associations of GAD65- and IA-2- autoantibodies with genetic risk markers in new-onset IDDM patients and their siblings. The Belgian diabetes registry. *Diabetes Care*. 1997;20(10):1547-1552.
26. Hagopian WA, Sanjeevi CB, Kockum I, et al. Glutamate decarboxylase-, insulin-, and islet cell-antibodies and HLA typing to detect diabetes in a general population-based study of Swedish children. *J Clin Invest*. 1995;95(4):1505-1511.
27. Mäkinen A, Härkönen T, Ilonen J, Knip M. Finnish pediatric diabetes register FPD. Characterization of the humoral immune response to islet antigen 2 in children with newly diagnosed type 1 diabetes. *Eur J Endocrinol*. 2008;159(1):19-26.
28. Lempainen J, Laine AP, Hammais A, et al. Non-HLA gene effects on the disease process of type 1 diabetes: from HLA susceptibility to overt disease. *J Autoimmun*. 2015;61:45-53.
29. Pugliese A, Zeller M, Fernandez A, et al. The insulin gene is transcribed in the human thymus and transcription levels correlated with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type 1 diabetes. *Nat Genet*. 1997;15(3):293-297.
30. Vafiadis P, Bennett ST, Todd JA, et al. Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus. *Nat Genet*. 1997;15(3):289-292.
31. Hagopian WA, Erlich H, Lernmark A, et al. The environmental determinants of diabetes in the young (TEDDY): genetic criteria and international diabetes risk screening of 421 000 infants. *Pediatr Diabetes*. 2011;12(8):733-743.
32. Burn GL, Svensson L, Sanchez-Blanco C, Saini M, Cope AP. Why is PTPN22 a good candidate susceptibility gene for autoimmune disease? *FEBS Lett*. 2011;585(23):3689-3698.
33. Hermann R, Lipponen K, Kiviniemi M, et al. Lymphoid tyrosine phosphatase (LYP/PTPN22) Arg620Trp variant regulates insulin autoimmunity and progression to type 1 diabetes. *Diabetologia*. 2006;49(6):1198-1208.
34. Bottini N, Peterson EJ. Tyrosine phosphatase PTPN22: multifunctional regulator of immune signaling, development, and disease. *Annu Rev Immunol*. 2014;32:83-119.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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APPENDIX A

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