Veijola Riitta (Orcid ID: 0000-0002-6557-270X)
Knip Mikael (Orcid ID: 0000-0003-0474-0033)

Heterogeneity in the presentation of clinical type 1 diabetes defined by the level of risk conferred by HLA class II genotypes

Antti-Mathias Taka[1,2], Taina Härkönen[1,2], Paula Vähäsalo[3,4], Johanna Lempainen[5,6,7], Riitta Veijola[3,4], Jorma Ilonen[5], Mikael Knip[1,8,9] and the Finnish Pediatric Diabetes Register*

[1]Pediatric Research Center, Children's Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

[2]Research Program for Clinical and Molecular Metabolism, Faculty of Medicine, University of Helsinki, Helsinki, Finland

[3]Department of Pediatrics, PEDEGO Research Unit, Medical Research Center, University of Oulu, Oulu, Finland

[4]Department of Children and Adolescents, Oulu University Hospital, Oulu, Finland

[5]Immunogenetics Laboratory, Institute of Biomedicine, University of Turku, Turku, Finland

[6]Department of Pediatrics, University of Turku and Turku University Hospital, Turku, Finland

[7]Clinical Microbiology, Turku University Hospital, Turku, Finland

[8]Tampere Center for Child Health Research, Tampere University Hospital, Tampere, Finland

A complete list of the investigators for the Finnish Pediatric Diabetes Register and Sample Repository is found in the Appendix.

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Corresponding author: Mikael Knip, MD, PhD
University of Helsinki
Biomedicum 1
Haartmaninkatu 8
00290 Helsinki
Finland
+358 50 448 7722
mikael.knip@helsinki.fi

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Abstract

Aims/hypothesis: The association between human leukocyte antigen (HLA) class II genotypes and susceptibility to type 1 diabetes (T1D) is well established. This study aimed at examining whether there are differences in the presentation of T1D depending on the HLA genotype.

Methods: We divided the study participants (N=5798) in the Finnish Pediatric Diabetes Register into two groups based on the T1D risk conferred by their HLA genotype (high and moderate risk genotypes, Group 1 vs. other genotypes, Group 2). We then examined differences in clinical, metabolic and immunological characteristics. Children included in the study were 0 to 14-year-old and diagnosed between January 2003 and December 2019.

Results: Participants in Group 1 were younger at the time of diagnosis (P<0.001) and had more frequently family members affected by T1D (P<0.001). Diabetic ketoacidosis (DKA) was more frequent among participants in Group 2 (P=0.014) who also had a longer duration of symptoms before diagnosis (P<0.001) and higher HbA1c (P=0.001) at diagnosis. The HLA genotype was not, however, directly related to the DKA frequency. The frequency of ICA (P<0.003), IAA (P<0.001) and IA-2A (P<0.001) was higher in Group 1 whereas GADA were more frequent (P<0.001) in Group 2. Group 1 had more participants with multiple autoantibodies (P=0.027) whereas antibody negativity was more frequent in Group 2 (P=0.003).

Conclusions: These findings indicate disease heterogeneity in relation to both clinical disease presentation and humoral autoimmunity, in particular. This heterogeneity is, at least partly, defined by HLA class II genotypes.

INTRODUCTION

Type 1 diabetes (T1D) is a polygenic disease; more than 60 loci have been noted to predispose to T1D (1). The most important and well known of these is the HLA region on chromosome 6p21. Genes encoding class II HLA-DR/DQ molecules account for approximately 50% of the genetic T1D susceptibility. Several studies on HLA haplotypes have shown that the highest risk is inflicted by heterozygosity for the DR4-DQ8 and DR3-DQ2 haplotypes, which also have a synergistic effect. Close to 90% of all people with T1D carry at least one of these haplotypes. Vice versa, some haplotypes have protective properties for example DRB1*15:01-DQA1*01:02- DQB1*06:02. (2–5) The HLA class II genotype has also been shown to affect the manifestation of T1D, as carriers of high-risk genotypes are diagnosed younger. (6–8)

In 2016, Ilonen et al. presented a risk classification for T1D based on the HLA-DR/DQ genotype frequencies observed in the Finnish population (9). This classification rests on disease risk associations of each haplotype taking into consideration the synergistic effects of the DR3-DQ2 and DR4-DQ8 haplotypes. Each haplotype is classified protective (strong or weak), neutral or susceptible (weak or strong). People are then divided into risk groups according to their genotypes on a scale from 0 to 5, in which 0 marks strong protection and 5 refers to the high-risk genotype.

In addition to the HLA genotype, islet autoantibodies can be used to predict disease risk (10,11). T1D is an autoimmune disease in which pancreatic beta cells are destroyed by the host's autoreactive T cells. The current concept is that islet autoantibodies are biomarkers reflecting the progression of the disease but do not directly participate in the destruction of beta cells. However, some studies suggest that autoantibodies may play a role in the disease process (12,13).

Islet cell antibodies (ICA) originally used to predict T1D were detected by the ability of the serum from the person assessed to stain pancreatic islets in an indirect immunofluorescence test and the test revealed multiple specificities of islet antigens. The ICA assay is largely replaced by specific islet autoantibodies detecting various beta-cell autoantigens including insulin autoantibodies (IAA), glutamic acid decarboxylase autoantibodies (GADA), islet antigen 2 autoantibodies (IA-2A) and zinc transporter 8 autoantibodies (ZnT8a) (14–16). Based on the number of positive antibodies and

the age at the time of seroconversion, predictions can be made for the likelihood of progression to symptomatic T1D (11,17–19). A combined analysis of three follow-up cohorts of newborns with an increased HLA associated genetic risk reported that positivity for multiple autoantibodies increase the risk of disease progression significantly. Risk for diabetes with no autoantibodies was 0.4%, with one autoantibody the risk was 14.5% and with multiple autoantibodies the risk was 69.7% over the next 10 years (10).

Furthermore, previous studies have indicated that childhood T1D is a heterogeneous disease. It has been suggested that there are two major forms distinguished by the initially detected autoantibody. In one form the first detectable autoantibody is IAA, which appears early in life, peaking before the age of 2 years. In the other form GADA is the first autoantibody to appear, peak of appearance being at the age of 4 to 5 years. The former form is also linked to faster disease progression. (20–23)

The DIPP study has since 1994 screened newborn infants for HLA-conferred susceptibility to T1D in three university hospitals in Finland and invited those with high- and moderate-risk genotypes to follow-up until the manifestation of T1D or the age of 15 years (24). The DIPP birth cohort screened covers more than 20% of all newborn infants in Finland since 1994. Ten percent of those screened are identified as risk individuals and this group includes approximately 60% of future cases diagnosed with T1D before adult age.

In the current study, we set out to assess whether the presentation of T1D is different in children with high- and moderate-risk HLA genotypes compared to children with other genotypes. We hypothesized that the group with higher risk genotypes would have more severe characteristics of T1D. We examined this on three fronts. First, we expected that the age at diagnosis would be younger in the group with higher risk HLA genotypes. Second, we hypothesized that the clinical manifestation of the disease would be more aggressive, which would show up in metabolic markers. Lastly, we expected that the group with higher risk for T1D would have higher islet autoantibody levels and higher frequency of autoantibody positivity.

METHODS

Subjects

The Finnish Pediatric Diabetes Register (FPDR) was initiated in 2002 to collect information and biological samples from children and adolescents with newly diagnosed diabetes as well as from their family members. All pediatric units taking care of newly diagnosed patients in Finland participate in the FPDR. The register has a wide coverage: more than 90% of all T1D cases are registered (25). Our data was derived from the register and included children diagnosed with T1D between January 2003 and December 2019. The inclusion criteria were (i) T1D, (ii) age under 15 years, (iii) HLA genotype data available and (iv) informed consent. The diagnosis was made based on the criteria set by the World Health Organization (26). Altogether, 5798 participants fulfilled the inclusion criteria. The study protocol was approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa.

We divided the participants into two groups according to the HLA risk classification presented by Ilonen et al. (13). Group 1 (N=3365) included children with moderately or highly increased risk of T1D with an odds ratio for T1D of 7.20 to 13.23 and an absolute risk of 4.4 to 9.5% in Finland. Group 2 (N=2433) included children with decreased risk, neutral risk or slightly increased risk, the odds ratios ranging from 0.02 to 1.94 and the absolute risk from 0.03 to 1.6%.

Measurements

Differences between the two groups in the characteristics of T1D were analyzed from three different viewpoints: demographic features, metabolic parameters at diagnosis and signs of humoral beta-cell autoimmunity. We assessed the following demographic parameters: sex, age, and family history of T1D. We examined a series of metabolic markers at the time of diagnosis: blood pH and HbA1c as well as plasma glucose and ß-hydroxybutyrate levels. Measuring of HbA1c was initiated only in 2013 which explains the lower number of participants with HbA1c values available. Diabetic ketoacidosis was defined as a pH < 7.30 and severe ketoacidosis as a pH < 7.10. Parents completed a structured questionnaire concerning the duration of symptoms

before the diagnosis. The alternatives were: no symptoms, symptoms for less than 1 week, symptoms for 1-2 weeks, 2-3 weeks, 3-4 weeks, or for more than 4 weeks.

HLA class II genotyping was performed with PCR-based lanthanide-labeled hybridization followed by time-resolved fluorometry detection as earlier described (9). The following islet autoantibodies were analyzed: ICA, IAA, IA-2A, GADA and ZnT8A. The biochemical autoantibodies (IAA, IA-2A, GADA and ZnT8A) were measured with specific radiobinding assays as previously described (27,28). ICA was analyzed with indirect immunofluorescense on human group 0 donor pancreas (29). The samples for autoantibody measurements were taken shortly after diagnosis, on average after 5.6 days (median 5 days). The thresholds for autoantibody positivity were 1.57 relative units (RU) for IAA, 0.77 RU for IA-2A, 5.36 RU for GADA, 0.50 RU for ZnT8A and 2.5 JDRF-units for ICA.

Data analysis

Frequencies were compared between the two groups with cross tabulation and Pearson's chi-squared test. Differences between non-parametric variables were tested with the Mann-Whitney U test whereas normally distributed variables were compared with Student's t test. A *P* value of 0.05 was used as the threshold for statistical significance. Logistic regression analysis was applied to assess which factors (T1D family history, duration of symptoms before diagnosis, HLA genotype) explained the frequency of diabetic ketoacidosis at the diagnosis of T1D. IBM SPSS software (version 24,IL, USA) was used for the statistical analyses.

RESULTS

Demographic and genetic information

Both groups included slightly more boys than girls: Group 1 55.9% and Group 2 56.8% (Table 1). There was no significant difference between the groups in the sex distribution (*P*=0.53). Children in Group 1 were younger at diagnosis of T1D (*P*<0.001) with a mean age of 7.9 years (range 0.3-15.0) whereas children in Group 2 were diagnosed at a mean age of 8.4 (range 0.5-15.0) years (Table 1).

Children in Group 1 had more frequently an affected family member [10.9% (95% CI 9.9-12.0) vs. 8.1% (95% CI 7.9-9.3); *P*<0.001]. Group 1 had both more affected fathers [6.2% (95% CI 5.4-7.1) vs 4.4% (95% CI 3.6-5.4); *P*=0.003] and mothers [3.5% (95% CI 2.9-4.2) vs 2.5% (95% CI 1.9-3.2); *P*=0.042]. There was no significant difference in the frequency of siblings affected by T1D (Table 1).

Clinical and metabolic markers

Ketoacidosis was more frequently present in Group 2 patients (20.8% vs 18.2%, *P*=0.014) (Table 2). The frequency of severe ketoacidosis also tended to be higher in Group 2 (P=0.064). We observed higher HbA1c levels in Group 2 at the time of diagnosis (*P*=0.001). There were also differences in the duration of symptoms (*P*<0.001). The patients in Group 1 had symptoms for a shorter time, which could be seen in relation to the frequencies of patients with no symptoms (2.3% vs 1.0%) and to symptoms for less than one week (23.6% vs 18.8%). Furthermore, Group 2 had more patients with symptoms for more than 4 weeks when compared to Group 1 (22.1% vs 17.4%).

The results of the logistic regression analysis showed that the lower frequency of diabetic ketoacidosis in Group 1 could be explained by a positive family history for T1D (*P*<0.001) and a short duration of symptoms before diagnosis (*P*<0.001) but not by the HLA genotype (P=0.12) (Table 3).

Humoral beta-cell autoimmunity

Higher frequencies were seen for IA-2A (*P*<0.001), ICA (*P*=0.003) and IAA (*P*<0.001) in Group 1 (Table 4). There was no significant difference regarding positivity for ZnT8A, whereas GADA-positivity was in contrast more frequent in Group 2 (*P*<0.001). ICA was the most frequently detected autoantibody with a prevalence of 92.3% in Group 1 and 90.1% in Group 2. IA-2A was the second most frequent autoantibody with detection rates of 78.9% and 68.6%. GADA and ZnT8A had similar frequencies of positivity: 64.0% and 69.5% for GADA, 68.8% and 70.0% for ZnT8A. The most infrequent autoantibody detected was IAA that was seen in only 57.8% of the participants in Group 1 and in 51.8% in Group 2.

We observed several differences in the number of positive autoantibodies. Autoantibody negativity was more frequent (*P*=0.003) in Group 2. Positivity for multiple autoantibodies was more common in Group 1. This was seen when analyzing the number of patients with all five positive autoantibodies (*P*=0.031) or with at least two (*P*=0.027), three (*P*=0.002) or four (*P*=0.007) positive autoantibodies. Similar findings were seen when analyzing only biochemical autoantibodies: positivity for multiple autoantibodies (*P*=0.002 for ≥ two, *P*=0.012 for ≥ three and *P*=0.045 for four positive autoantibodies) was more prevalent in Group 1 and having no positive (*P*=0.032) or only one positive autoantibody (*P*=0.030) was more frequent in Group 2. Levels of IAA (*P*=0.027) and IA-2A (*P*<0.001) were significantly higher in Group 1, whereas ZnT8A levels were higher in Group 2 (*P*=0.002). There were no significant differences in the levels of ICA or GADA between the two groups.

DISCUSSION

Our hypothesis was that children carrying moderate or high-risk HLA class II genotypes would be diagnosed with type 1 diabetes at a younger age and that the disease would be more aggressive. Our results confirmed our hypothesis concerning the age at diagnosis but not regarding the metabolic characteristics. In contrast, we observed a more aggressive disease presentation in children with other HLA genotypes, as we saw more patients with ketoacidosis and higher HbA1c concentrations in Group 2. Furthermore, children in Group 1 had symptoms for a shorter time before the diagnosis and those in Group 2 had more often symptoms for more than 4 weeks. The observations of higher HbA1c levels and longer duration of the preceding symptomatic period in Group 2 are in line, as the HbA1c reflects the child's glucose levels over the past 2-3 months (26,30). Having a family member affected by T1D, younger age at diagnosis and higher risk HLA class II genotype, all of which seen in Group 1, have previously been associated with decreased risk of DKA at diagnosis (31,32). Lower risk of DKA due to an affected first-degree relative (FDR) is expected since such families are familiar with the symptoms of T1D and therefore recognize the need for seeking medical attention earlier. In addition, a small proportion of the patients in Group 1 participate in the prospective DIPP study. Such participation has been shown to decrease the

frequency of ketoacidosis at the time of diagnosis of T1D, whereas genetic screening has not (33). The results of the logistic regression analysis indicate that the HLA genotype does not have a direct effect on the incidence of ketoacidosis.

It has been reported that HLA class II genes are not involved in the disease progression from the appearance of autoantibodies to overt T1D. Instead, several studies support a role of HLA class I and other genes in the progression from autoantibody positivity to T1D (9,34–36). This is theoretically supported by what is known about the mechanisms of immune function: any autoimmune reaction begins when CD4+ T cells recognize antigens presented by HLA class II molecules and help production of autoantibodies by B cells. Cytotoxic CD8+ T cells in turn recognize antigens presented by HLA class I molecules. The destruction of beta cells in pancreas is carried out by such cytotoxic T cells, resulting in disease progression and finally to diagnosis of clinical T1D. It has, however, been implicated that HLA class I genotypes may affect progression to T1D partly depending on the HLA class II genotype due to linkage disequilibrium (37). Our findings indicate that HLA class II is associated with disease progression, as the class II genotypes are related to a younger at diagnosis of clinical diabetes as well as autoantibody frequencies and levels. We do not have information on the HLA class I genotypes in our study subjects. Accordingly it is possible to only speculate whether the association of HLA class II genotypes are independent or whether the association is partly mediated through linkage disequilibrium with HLA class I genotypes.

As mentioned earlier, it has been suggested that T1D can be divided in two forms (endotypes) depending on the first autoantibody detected (IAA or GADA) in the asymptomatic period preceding the diagnosis of symptomatic disease. The form in which IAA appear first, is associated with faster disease progression. We could see significant (P<0.001) differences in the frequencies of IAA and GADA between Group 1 and 2. Group 1, with higher risk HLA genotypes, tested more frequently positive for IAA (57.8% vs 51.8%) whereas GADA positivity was more frequent in Group 2 (69.5% vs 64.0%). Among the IAA-positive participants the IAA levels were also higher in Group 1 (P=0.027). We do not have information on the order in which autoantibodies appeared during the preclinical phase, but these results indicate that Group 1 is characterized by features reflecting the

"IAA first" endotype, whereas the features of the "GADA first" endotype are more frequent in Group 2.

Male gender was slightly more prevalent both in Group 1 and Group 2. This is in line with previous studies reporting that male gender is associated with a higher incidence of T1D particularly in Caucasian populations with high disease rates (38). Our findings are also in line with previous studies on the differences of T1D risk depending on the affected family member, as the frequency of paternal T1D has been observed to be almost two times higher than that of maternal T1D (39,40). Furthermore, an increased frequency of affected family members in participants with higher risk HLA genotypes seen in our study has been reported earlier (32,41).

Significant differences in islet autoantibody frequencies were seen. As discussed earlier, seroconversion to positivity for multiple islet autoantibodies is highly predictive of T1D. In the current study more than 90% of all participants tested positive for multiple autoantibodies, which were more prevalent in Group 1, whereas autoantibody negativity was more frequent in Group 2. These findings reflect a more intensive beta-cell autoimmunity in Group 1. There were also differences in the frequency of various autoantibodies in the two groups studied. IAA and IA-2A are known to be strongly associated with the HLA DR4-DQ8 haplotype, especially when including the DRB1*04:01 allele, which is the high-risk DRB1 haplotype, whereas GADA is associated with the weaker DR3-DQ2 susceptibility haplotype (42). Indeed, among our study subjects IAA, ICA and IA-2A were more frequent in Group 1 carrying high or moderate risk genotypes for T1D whereas GADA were more frequent in Group 2 with lower HLA-conferred risk for T1D. The majority of Group 1 children carry moderate risk genotypes which comprise mainly genotypes where the high risk associated DRB1*04:01 positive DR4-DQ8 haplotype is combined with a "neutral" haplotype whereas the majority of Group 2 comprises "slightly increased risk" genotypes where DR3-DQ2 or DR4-DQ8 haplotype including the lower risk DRB1*04:04 allele combine with "neutral" haplotypes (9). Finally, differences in islet autoantibody levels were seen for IAA, IA-2A and ZnT8A between the two groups. Higher IA-2A levels in Group 1 could be an indicator of a faster disease progression since IA-2A has been linked to rapid beta-cell destruction and thus to eralier manifestation of overt disease (17,43).

Our results indicate that there are differences in the characteristics of patients with newly-diagnosed T1D depending on HLA class II genotype. Patients with high or moderate risk genotypes are younger at diagnosis and have more family members affected by T1D than those with other HLA genotypes. In addition, they have a somewhat lower frequency of ketoacidosis, a lower HbA1c, and a shorter duration of symptoms at diagnosis. Patients with higher risk HLA genotypes also have a higher frequency of IAA, IA-2A, and ICA and higher levels of IAA and IA-2A but a reduced frequency of GADA and lower levels of ZnT8A when compared to those with other genotypes.

We feel that the sample size of the current study is a major strength. The information was derived from a nationwide study among all newly-diagnosed children in Finland that is the country with highest incidence of T1D in the world. The cross-sectional design of the study is a limitation, as we have no data on the natural course of T1D before or beyond the time of diagnosis. In addition, a relatively high proportion of missing data for a few metabolic markers (i.e. HbA1c and β-hydroxybutyrate) weakens the reliability of findings related to such markers. Additional data on HLA class I and non-HLA genetic markers could also have been informative.

In conclusion, our results support previous findings on the heterogeneity of T1D. Heterogeneity was detected in relation to age at diagnosis, humoral autoimmunity and also in metabolic characteristics at the time of diagnosis of T1D. More research is needed in this field, and knowledge of different disease pathways could open up novel approaches to study the association between genetic factors and exogenous elements involved in the development of T1D.

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Author contributions

A-M.T. analyzed the data and wrote the first version of the manuscript. T.H. and P.V. were responsible for the autoantibody analyses, reviewed the manuscript and contributed to the discussion. J.I. was in charge of the HLA genotyping, reviewed the manuscript and contributed to the discussion. R.V. was responsible for the ICA laboratory, reviewed the manuscript and contributed to the discussion. M.K. planned the study, reviewed the manuscript and contributed to the discussion. M.K. is the guarantor of this work and, as such, had the full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data

ORCID

Antti-Mathias Taka https://orcid.org/0000-0002-1970-4978

Paula Vähäsalo http://orcid.org/0000-0002-2712-3903

Riitta Veijola http://orcid.org/0000-0002-6557-270X

Jorma Ilonen http://orcid.org/0000-0002-9973-2062

Mikael Knip http://orcid.org/0000-0003-0474-0033

APPENDIX

The Finnish Pediatric Diabetes Register comprises the following investigators:

***Principal Investigator*:** Mikael Knip (Children's Hospital, Helsinki University Hospital)

Steering Committee: Per-Henrik Groop (Folkhälsan Research Center), Jorma Ilonen (Immunogenetics Laboratory, University of Turku), Timo Otonkoski (Children's Hospital, Helsinki University Hospital), Riitta Veijola (Department of Pediatrics, Oulu University Hospital).

Locally responsible investigators:

Alar Abram (Department of Pediatrics, Kanta-Häme Central Hospital), Henrikka Aito (Department of Pediatrics, HUS Porvoo Hospital), Ivan Arkhipov (Department of Pediatrics, Mehiläinen Länsi-Pohja Central Hospital), Elina Blanco-Sequeiros (Department of Pediatrics, Central Ostrobothnia Central Hospital), Jonas Bondestam (Department of Pediatrics, HUS Lohja Hospital), Markus Granholm (Department of Pediatrics, Jakobstad Hospital), Maarit Haapalehto-Ikonen (Department of Pediatrics, Rauma Hospital), Torsten Horn (Department of Pediatrics, Central Hospital of Central Finland), Hanna Huopio (Department of Pediatrics, Kuopio University Hospital), Joakim Janer (Department of Pediatrics, HUS Raasepori Hospital), Christian Johansson (Department of Pediatrics, Åland Central Hospital), Liisa Kalliokoski (Department of Pediatrics, Kainuu Central Hospital), Päivi Keskinen (Department of Pediatrics, Tampere University Hospital), Anne Kinnala (Department of Pediatrics, Turku University Central Hospital), Maarit Korteniemi (Department of Pediatrics, Central Hospital of Lapland), Hanne Laakkonen (Department of Pediatrics, HUS Hyvinkää Hospital), Jyrki Lähde (Department of Pediatrics, Satakunta Central Hospital), Päivi Miettinen (HUS New Children's Hospital). Päivi Nykänen (Department of Pediatrics, Mikkeli Central Hospital), Erik Popov (Department of Pediatrics, Vaasa Central Hospital), Mari Pulkkinen (Department of Pediatrics, HUS Jorvi Hospital), Maria Salonen (Department of Pediatrics, Kymenlaakso Central Hospital), Pia Salonen (Department of Pediatrics, Päijät-Häme Central Hospital), Juhani Sankala (Department of Pediatrics, Savonlinna Central Hospital), Virpi Sidoroff (Department of Pediatrics, North Karelia Central Hospital), Anne-Maarit Suomi (Department of

Pediatrics, South Ostrobothnia Central Hospital, Tuula Tiainen (Department of Pediatrics, South Karelia Central Hospital), Riitta Veijola (Department of Pediatrics, Oulu University Hospital)

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TABLE 1 Demographic and genetic factors in children and adolescents with newly-diagnosed type 1 diabetes. Patients carrying high and moderate risk HLA genotype (Group 1) and other HLA genotypes (Group 2) are compared. Proportions of children with different class II HLA genotypes are given for both groups.

Variable	N	Group 1, N= 3365	Group 2, N= 2433	*P* value
Demographics	5798			
Sex, male, %		55.9	56.8	0.53
Age at diagnosis, years, mean +/- SD		7.9 +/- 3.91	8.4 +/- 3.82	**<0.001**
T1D in family	5798			
Father with T1D, %		6.2	4.4	**0.003**
Mother with T1D, %		3.5	2.5	**0.042**
Sibling with T1D, %		1.9	1.4	0.20
First-degree relative with T1D, %		10.9	8.1	**<0.001**
HLA risk group	5798			
Strongly decreased risk, %			0.7	
Slightly decreased risk, %			2.2	
Neutral, %			15.6	
Slightly increased risk, %			23.5	-
Moderately increased risk, %		36.7		
High risk, %		21.4		

TABLE 2 Comparison of clinical and metabolic markers between children and adolescents with newly-diagnosed type 1 diabetes carrying high and moderate risk HLA genotype (Group 1) and other HLA genotypes (Group 2).

Variable	N	Group 1, N= 3365	Group 2, N= 2433	*P* value
Metabolic markers				
Ketoacidosis, %	5597	18.2	20.8	**0.014**
Severe ketoacidosis, %	5597	4.6	5.8	0.064
pH, median (range)	5597	7.38 (6.69-7.57)	7.38 (4.60-7.54)	0.71
β-h§ydroxybutyrate, mmol/l, median (range)	5108	1.7 (0-52.0)	1.9 (0-18.0)	0.060
Plasma glucose, mmol/l, median (range)	5646	23.8 (3.2-94.6)	24.0 (4.3-95.6)	0.73
HbA1c, mmol/l, median (range)	1784	90.0 (33.0-176.0)	95.0 (30.0-303.3)	**0.001**
Duration of symptoms, %	5312			**<0.001**
No symptoms		2.3	1.0	
< 1 week		23.6	18.8	
1-2 weeks		31.6	32.3	
2-3 weeks		11.4	11.5	
3-4 weeks		13.6	14.3	
> 4 weeks		17.4	22.1	

TABLE 3 Logistic regression of children and adolescents with newly-diagnosed type 1 diabetes. Associations between an affected first-degree relative (FDR), lower risk HLA class II genotype and duration of symptoms and the incidence of ketoacidosis. Coefficient for constant (B), standard error (SE), Wald chi-square (Wald), *P* value, exponentation of the B coefficient (Exp (B)) and confidence intervals (CI) calculated.

	B	SE	Wald	*P* value	Exp (B)	95% CI
Affected FDR	-0.97	0.16	34.55	**<0.001**	0.38	0.28-0.53
HLA risk class (1=Group 1, 2=Group 2)	0.11	0.071	2.49	0.12	1.12	0.97-1.29
Duration of symptoms						
No symptoms	-2.39	0.72	11.01	**0.001**	0.092	0.022-0.38
< 1 week	-0.25	0.11	5.06	**0.024**	0.78	0.63-0.97
1-2 weeks	-0.12	0.098	1.41	0.24	0.89	0.74-1.08
2-3 weeks	-0.12	0.13	0.89	0.35	0.89	0.69-1.14
3-4 weeks	-0.17	0.12	1.98	0.16	0.84	0.67-1.07
> 4 weeks			15.29	0.009		

TABLE 4 Comparison of immunological markers between children and adolescents with newly-diagnosed type 1 diabetes carrying high and moderate risk HLA genotype (Group 1) and other HLA genotypes (Group 2).

Variable	N	Group 1, N= 3365	Group 2, N= 2433	*P* value
Frequency and levels of autoantibodies				
IAA positivity, %	5798	57.8	51.8	**<0.001**
IAA, RU, median (range)	3206	7.31 (1.57-7809.00)	6.56 (1.58-440.54)	**0.027**
GADA positivity, %	5798	64.0	69.5	**<0.001**
GADA, RU, median (ran	3844	35.05 (5.36-24849.00)	36.97 (5.43-7514.00)	0.16
IA-2A positivity, %	5798	78.9	68.6	**<0.001**
IA-2A, RU, median (range)	4325	107.85 (0.78-453.68)	96.45 (0.79-553.32)	**<0.001**
ZnT8A positivity, %	5798	68.8	70.0	0.34
ZnT8A, RU, median (range)	4018	10.44 (0.51-1201.90)	13.48 (0.51-263.16)	**0.002**
ICA positivity, %	5798	92.3	90.1	**0.003**
ICA, RU, median, (range)	5297	64 (3-5120)	65 (3-4096)	0.82
Number of autoantibodies	5798			
0 positive, %		1.5	2.6	**0.003**
1 positive, %		4.6	5.0	0.55
2 positive, %		10.3	12.0	**0.047**
3 positive, %		23.9	24.3	0.75
4 positive, %		33.6	32.5	0.41
5 positive, %		26.1	23.6	**0.031**
≥ 2 positive, %		93.9	92.4	**0.027**
≥ 3 positive, %		83.6	80.4	**0.002**
≥ 4 positive, %		59.7	56.1	**0.007**
Number of autoantibodies, ICA excluded	5798			
0 positive, %		3.0	4.1	**0.032**
1 positive, %		11.2	13.1	**0.030**
2 positive, %		25.2	25.5	0.80
3 positive, %		34.3	33.3	0.46
4 positive, %		26.3	23.9	**0.045**
≥ 2 positive, %		85.8	82.8	**0.002**
≥ 3 positive, %		60.6	57.3	**0.012**