WITHDRAWN:
Relative Frequency of Islet Autoimmunity in Children and Adolescents with Autoimmune Thyroid Disease

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Research Article

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EDITORIAL NOTE:

The full text of this preprint has been withdrawn by the authors while they make corrections to the work. Therefore, the authors do not wish this work to be cited as a reference. Questions should be directed to the corresponding author.

Abstract

The present study aims to investigate islet autoimmunity and susceptibility to type 1 diabetes (T1D) in children/adolescents with autoimmune thyroid disease (AITD), and family members of AITD patients with islet autoimmunity. Islet-cell cytoplasmic, glutamic-acid decarboxylase and tyrosine-phosphatase autoantibodies were measured in 161 AITD patients [(127 with autoimmune thyroiditis (AT); 34 with Graves' disease (GD)], 20 family members of AITD patients with islet autoimmunity, and 155 age-matched controls. Islet autoimmunity was found in 10.6% of AITD patients, significantly more frequent than in controls (1.9%; p=0.002). Higher prevalence of islet autoantibodies was found in females with AITD (p=0.011) but not in males (p=0.16) as well as in AT (p=0.013) but not GD patients (p=0.19), compared to corresponding controls. Two or three islet autoantibodies were found concurrently in six AITD patients with islet autoimmunity. They all developed T1D and had significantly higher islet autoantibodies titers (p=0.01) compared to AITD patients with single islet autoantibodies but normal glucose metabolism. T1D was found in 3.7% of AITD patients compared to 0.2% in age-matched, general Croatian population. Islet autoantibodies were found in 5/20 family members of AITD patients with islet autoimmunity, among which two developed T1D. None of the controls was positive to more than one islet autoantibodies or developed T1D.

Conclusion: Children/adolescents with AITD (particularly females and patients with AT) represent a risk group for islet autoimmunity and T1D, as well as family members of AITD patients with positive islet autoantibodies, but last observation must be examined in a larger number of patients.

What Is Known

- Two most common autoimmune endocrine diseases diabetes mellitus type 1 (T1D) and autoimmune thyroid diseases (AITD) - autoimmune thyroiditis and Graves' disease, are often found in same patient and/or within the same families.
- Thyroid autoimmunity was widely studied in T1D patients, but few studies examined islet autoantibodies and risk of development of T1D among AITD.

What is New

- This is the first comprehensive study which included children/adolescents with both AITDs (autoimmune thyroiditis and Graves' disease) and in which islet cell autoimmunity was estimated measuring three islet autoantibodies.
- Observed relative frequency of T1D development in AITD patients was much higher as compared to that in general Croatian population (3.7% vs 0.2%).
- In addition, the study is the first to evaluate islet autoimmunity and glucose metabolism in family members of AITD patients with islet autoantibodies, indicating their increased risk for developing T1D, but this observation must be confirmed in a larger number of patients.

Introduction

Autoimmune endocrine diseases are organ-specific diseases in which the immune response target organs are endocrine glands. Most common of these diseases are diabetes mellitus type 1 (T1D), and two autoimmune thyroid diseases (AITD): autoimmune thyroiditis (AT) and Graves' disease (GD). Of all autoimmune endocrinopathies that occur simultaneously, AITD and T1D are far more often found in the same person or within the same family [1,2]. This phenotype is classified as a variant of the autoimmune polyglandular syndrome type 3 (APS3v) [3,4].

Thyroid autoimmunity was widely studied in T1D, and thyroid autoantibodies (AAb) were found in about 8-44% of patients with T1D [5], while 50% of these patients develop clinical form of AITD [5]. On the other hand, few studies examined islet autoimmunity and risk for development of T1D among AITD patients, and only four of them were conducted in children and adolescents [6-9]. One study was performed in children with AT and GD [6] and three others in children with AT [7-9]. Among studies performed in adults, some were exclusively carried out in patients with AT [10,11] or GD [12,13] while others included patients with both conditions [14-18]. Islet autoimmunity was assessed by measuring different diabetes-associated AAb, as serological markers of β-cell autoimmunity, which included glutamic acid decarboxylase (GAD), islet cell cytoplasmic AAb (ICA), tyrosine-phosphatase (IA2), insulin (IAA), proinsulin and zinc-transporter AAb. The presence of these antibodies is age dependent, with IAA and IA-2 more commonly seen in children less than 10 years or age, while GAD are associated with older age and with female gender [19,20].

The aim of this study was to assess the relative frequency of humoral markers of autoimmunity to islet cells (ICA, GAD, IA2) in children and adolescents with AITD, and among AT and GD patients, separately. Additionally, we wanted to assess the relative frequency of T1D in the same group of patients as well as in family members (parents, siblings) of patients with AITD and islet autoimmunity. In AITD patients and family members who tested positive for islet AAb, glucose metabolism was assessed in order to evaluate for T1D.

Patients And Methods

Patients

The study included 161 patients with AITD divided into two groups [127 with AT (29 males and 98 females, aged 4.17-19.0 years) and 34 with GD (7 males and 27 females, aged 6.5-21.9 years)] and 20 family members (18 parents and 2 siblings) of patients with AITD and positive islet AAb. All patients were treated at the Department of Pediatric Endocrinology and Diabetes, University Hospital Center Zagreb, in the period from June 2012 to June 2018. Patients affected by any syndromic or other known genetic disease (e.g. Turner, Down or Klinefelter syndrome, as well as polyglandular syndromes) were excluded from the study.

The control group consisted of 155 patients (52 males and 103 females, aged 4.0-21.5 years), admitted to the Department of Pediatrics, University Hospital Centre Zagreb for evaluation of other non-chronic diseases, whose clinical history was negative for thyroid autoimmunity and other autoimmune diseases,

and who had no family history of T1D and AITD.

The diagnosis of AT was based on the presence of elevated titers of AAb against thyroid peroxidase (TPO) and/or thyroglobulin (Tg) and thyroid ultrasound examination consistent with this diagnosis. The diagnosis of GD was based on clinical and biochemical findings of hyperthyroidism and thyroid ultrasound examination.

The University Hospital Center Zagreb Ethics Committee approved the study protocol in accordance with the Declaration of Helsinki, and informed consents were obtained from all participants and/or their parents.

Parameters in study

In both patient and control groups the titers of Tg, TPO AAb, and islet AAb (GAD, IA2 and ICA), were assessed at the time of evaluation.

Islet AAb were also measured in 20 family members (10 mothers, 8 fathers and 2 sisters) of 10 AITD patients with islet autoimmunity. In 16 patients with AITD and islet autoimmunity, four of their family members with islet autoimmunity and in three control subjects with islet autoimmunity, glucose metabolism was evaluated with oral glucose tolerance test (OGTT) and HbA1c (using the immunoassay method in Siemens A1c Vantage Analyzer, Siemens Healthcare GmbH, Erlangen, Germany) according to ISPAD criteria [21].

Determinations of GAD and IA2 AAb were performed by commercial ELISA kits (EUROIMMUN, Germany). In 2010 Clinical Institute of Laboratory Diagnosis, University Hospital Merkur, Zagreb participated in Diabetes Antibody Standardization Program – DASP. Sensitivities and specificities were 88% and 94%, respectively, for GAD, 72% and 99%, respectively, for IA-2 AAb. Cut-off for positive results was set at 5 Units/mL for GAD and 10 Units/mL for IA2 antibodies [22].

Detection of ICA autoantibodies was performed by indirect immunofluorescence. Scores of fluorescence intensities were then calculated into Juvenile Diabetes Foundation units (JDF). Results >5 JDF units were considered positive. ICA assays were validated by repeated participation in immunology of diabetes workshops and proficiency testing programs of the University of Florida (Gainesville, FL) with >95% sensitivity, specificity, consistency, and validity [23]. Quality of our performance is validated by yearly continuous participation in Instand EQA schemes. The cut-off values of positivity for TPO and Tg AAb were 20.0 Units/mL and 60.0 Units/mL, respectively. The reference ranges and cut-off values for the enzyme-linked immunosorbent assay (ELISA Brahms GmbH, Henningsdorf, Germany) methods were provided by the manufacturer and results higher than cut-off values set by the manufacturer were considered positive.

Statistical analysis

Data are presented in tables using descriptive statistics (frequencies, means and standard deviations). Comparison among the patient groups was done using the appropriate tests, depending on the data type and their distribution (Chi square test, Fisher exact test and the Mann-Whitney U test for unpaired data). Statistical Package for the Social Sciences version 21.0 (IBM SPSS Statistics, USA) was used for calculations and $p<0.05$ was considered significant.

Results

Islet AAb and glucose metabolism in AITD patients

Islet autoimmunity was significantly more frequent in patients with AITD (10.6%) than in the control group (1.9%; p=0.002). The frequency was significantly higher only in AT patients (11.8%; p=0.001), but not in GD patients (5.9%, p=0.19), as compared to controls (Table 1). AITD patients with islet autoimmunity were slightly younger at the time of evaluation (median 11.6 years), than those without islet autoimmunity (median 12.8 years) but the difference was not statistically significant (Mann-Whitney test: U=1071, z =1.26, p=0.21).

Relative frequencies of all islet AAb were significantly higher in AITD patients compared to controls (ICA p=0.04; GAD p=0.002; IA-2 p=0.02, Table 2).

The clinical and laboratory characteristics of patients with AITD and islet autoimmunity are summarized in Table 3. Three out of 17 AITD patients with islet autoimmunity (patients #1-3), were positive for three islet AAb and additional 3 patients (patients #4-6) were positive for two islet AAb. In contrast, none of the control subjects was positive for more than one islet AAb (Table 2 and 3).

There was no statistically significant difference in frequency of islet autoimmunity between sexes (5.6% males and 12% females with AITD; p=0.27). Statistically significant difference in total islet autoimmunity was found in females with AITD compared to females in control group (12% vs 2.9%, p=0.011), but not among males with AITD (5.9% vs. 0%, p=0.16) as compared to males in control group.

When analyzed separately, statistically significant differences were found in frequencies of GAD (7.2%) and IA-2 AAb (5.6%) in females with AITD compared to females in control group (0%; p=0.005 and 1%; p=0.06, respectively). Statistically significant differences were not found in frequencies of ICA AAb in females with AITD (6.4%) compared to females in control group (1.9%; p=0.10), as well as in frequencies of all three AAb in males with AITD (ICA 2.8%; GAD 2.8%; IA-2 0%) compared to males in control group (0%).

At the time of evaluation, T1D was diagnosed in 1/16 AITD patients with islet autoimmunity (patient #1, HbA1c 9.8%; 83.6 mmol/mol) (Table 3) and one patient had impaired glucose tolerance and normal HbAlc (patient #3, Table 3). The remaining 14 patients had normal blood glucose levels in OGTT and

normal values of HbA1c. During the 6-year follow-up, 5/16 AITD patients with islet AAb (4 females and 1 male, patients #2-6; Table 3) developed T1D. Patient #17 was lost to follow up and her glucose metabolism was not investigated. Six patients who developed T1D were all tested positive for two or three AAb. All these patients were younger than 15 years of age at the time of T1D diagnosis. Relative frequency of T1D in our AITD patient cohort was 3.7%, while in patients younger than 15 years of age, the relative frequency was 4.8%. According to Croatian registry of diabetes in children and adolescents, prevalence of T1D in general age-matched Croatian population is 0.2% (*unpublished data*).

AITD patients with islet autoimmunity who developed T1D had significantly higher titers of islet AAb in comparison to AITD patients with islet autoimmunity and normal glucose metabolism (Mann-Whitney test, N= 16, Table 3: ICA – U = 6.5; z=-2.50; p=0.01; GAD– U = 7.0; z=-2.44 p= 0.02; IA2– U = 7.5; z=2.51; p=0.01). None of the patients in the control group with positive islet AAb developed T1D during the follow up.

Islet autoantibodies and glucose metabolism in family members of patients with AITD and islet autoimmunity

Positive islet AAb were found in 5/20 family members of patients with AITD and islet autoimmunity (3/9 fathers and 2/10 mothers). Mother of patient #1 was positive to three islet AAb and was diagnosed with T1D at the age of 19. Other four parents (#2b, 4b, 11a and 11b) with positive islet AAb had normal levels of glucose in OGTT and normal values of HbA1c at the time of evaluation. After the first evaluation, HbA1c was measured every 6-8 months. Father of patient #2, with positive GAD AAb and ICA, developed T1D after six years of follow-up at the age of 46 (Table 4).

Antithyroid AAb in AITD patients

AITD patients with islet autoimmunity had higher TPO and Tg AAb titers compared to AITD patients without islet autoimmunity, but the difference was not statistically significant (Mann-Whitney test: Tg– U=1126, z –-0.28; p=0.78; TPO – U=1768, z -2.05; p= 0.04)

Discussion

In this study of islet autoimmunity, we analyzed three islet AAb (ICA, GAD and IA2) in the group of children and adolescents with AITD (AT and GD) and in the control group. We found that 10.6% of AITD patients were positive for one or more islet AAb, which was significantly more than in controls (1.9%, p=0.002). This difference was entirely due to antibodies found in AT patients, in comparison to controls (11.8% vs 1.9%; p=0.001), as we found no significant difference in islet autoimmunity between the GD patients and control group. All of our patients were positive for thyroid AAb prior to inclusion in the study which allowed us to select and follow the patients that developed thyroid autoimmunity before the onset of T1D. When analyzing islet AAb separately, all three AAb were significantly more frequent in AITD patients compared to the control group (ICA p=0.002, IA-2 p= 0.001), and GAD AAb were found only in the AITD patients, but not in controls.

Few studies reported the frequency of ICA autoimmunity in patients with AITD and only four of them included children and adolescents [6-9]. Bright et al. found ICA AAb in 2.3% of children with AITD compared to 0% of controls [6]. In studies conducted in adult AITD patients, ICA positivity ranged from 0 - 4.9% [12,13,15,17].

Only one study evaluated frequency of IA2 AAb in children with AT (but not with GD) and found them to be more common than in control subjects (3.39% vs. 1.16%, p=0.012) [7], as was confirmed in our study. In one study conducted in adult patients, IA2 AAb were found more frequently in patients with AT as well as GD [18].

GAD positivity was assessed in three studies conducted in children with AT [7,9]. In two of them [8,9], GAD AAb were found significantly more often in children with AT than in controls (9.8-10.6% vs. 0-3.3%, p= 0.003 and p=0.036, respectively), as was confirmed in our study. However, in the study by Pilia et al. the difference was not significant [7]. Several studies analyzed GAD autoimmunity in adult patients with AT [10,11,14-16] and GD [12,13,15,16]. Relative frequency of GAD AAb ranged from 3.4-6.6% [10,11,14-16] in patients with AT, and although the positive correlation between GAD AAb and AT was found in some of the studies [15], it was not always statistically significant [16]. In adult GD patients, GAD AAb were found in 6.1-13% of patients, [12,13,15,16], significantly more common compared to controls in some of the studies [15,16]. However, control subjects were not always included in the evaluation [10-14].

Recently, islet AAb and thyroid autoimmunity were analyzed in children [24,25] and in adult patients [24], but these results cannot be compared to results from our study because they did not differentiate patients with AITD and risk for development of T1D from those that already had T1D.

We did not find significant differences in islet autoimmunity between males and females with AITD. However, females with AITD were positive to islet AAb, particularly to GAD and IA-2 AAb, significantly more often than females in control group, but this difference was not observed for ICA AAb. On the other hand, in males with AITD, we did not find any difference in islet autoimmunity compared to controls. As thyroid autoimmunity and AITD are more common in females, female gender was proposed as a risk factor for positive association between islet autoimmunity and thyroid autoimmunity [26].

In 16 patients with AITD who were positive for islet AAb, we wanted to assess the susceptibility for T1D development. One patient was diagnosed with T1D upon initial evaluation and five developed T1D during the follow up period of six years (Table 3). However, we cannot exclude that more patients would develop diabetes if the follow-up was longer. In the study of Bright et al. [6] one of two children with AT and positive ICA AAb developed diabetes after one year, and two children with AT and negative ICA AAb after four and six years, respectively. Pilia et al. [7] reported that over two years of follow-up, 2/19 children with AT and islet autoimmunity developed T1D (one positive to GAD AAb and the other to GAD and IA2 AAb). Lethagen et al. [10] found reduced ability of insulin secretion in GAD AAb positive AT patients and concluded that GAD AAb may be a marker of subclinical insulitis. During the follow-up of 4 years, 2/15 of their

GAD AAb positive patients (compared to 11/426 GAD AAb negative patients) were diagnosed with diabetes (p=0.08) [10]. Hallengren et al. [13] followed nine GAD AAb positive patients (two of them also ICA positive) for 27-70 months. One patient, who was positive for both islet AAb, developed diabetes. Maugendre et al. [12], found high frequency of GAD AAb (16/150 GD patients) but a low progression towards diabetes (only one patient). Aksoy et al. [11] studied insulin sensitivity and secretion patterns in GAD AAb positive and GAD AAb negative AT patients and concluded that it does not seem likely that presence of GAD AAb *per se* is associated with disturbance in glucose metabolism. Significant relationship between the higher titer of GAD AAb and abnormalities of glucose metabolism was found by Marhawa et al. [8] and Moriguchi et.al [16], but Kawasaki et al [15] did not report similar findings. In our study, AITD patients who developed T1D had significantly higher titers of GAD AAb (p= 0.02), compared to AITD patients with islet autoimmunity and normal glucose metabolism. Moreover, we noticed significantly higher titers of ICA and IA-2 AAb (both p=0.01) in this patient group.

We further measured TPO and Tg AAb and found higher titers in AITD patients with islet autoimmunity, compared to AITD patients without islet autoimmunity, although the difference was not statistically significant. Marwacha et al. [8] found that GAD AAb levels increased with increasing titer of anti-TPO AAb, but Yamaguchi et al. [17] and Kawasaki et al. [15] did not find correlations for the titer level of ICA [17] or IAA and GAD AAb [15].

Also, the observed relative frequency of T1D development in our patients with AITD was compared to that in Croatian general population. In our cohort T1D was found in 3.7% of AITD patients, much more frequently than in general population in the same age groups (0.2%) [27*; Croatian registry of diabetes in children and adolescents, unpublished data*].

Islet autoimmunity and susceptibility to T1D was also investigated in 20 first-degree family members of patients with AITD and positive islet AAb. Positive islet AAb were found in 5/20 family members (3/9 fathers and 2/10 mothers). One of the mothers was already diagnosed with T1D, and one father developed T1D during the follow-up, suggesting that family members of patients affected with AITD and islet autoimmunity might have higher risk for development of T1D. However, we did not test family members of AITD patients without positive islet AAb. It would be necessary to confirm these results on larger number of family members of patients with AITD, both those with and without islet autoimmunity, in order to determine the risk for glucose metabolism impairment in relatives of patients with AITD.

As development of T1D cannot be prevented or delayed, the clinical significance of screening for islet AAb and continuous follow-up of antibody positive patients is uncertain [28]. However, as new studies found decreased rate of diabetic ketoacidosis in individuals screened for islet AAb [29], it might be important to identify groups at risk for development of T1D. Nevertheless, some authors concluded that screening for T1D risk to reduce diabetic ketoacidosis is not economically viable [30].

In conclusion, we found that children and adolescents with AITD, in particular females with AT and patients with higher titer of two or more islet AAb, but also family members of AITD patients with positive islet AAb, have an increased risk for developing T1D. Prospective long-term studies on a larger number of subjects are required to examine the factors responsible for islet cell destruction, insulin deficiency and evolution towards diabetes in patients with AITD. Patients with GD are particularly underrepresented and not well studied and it would be of importance to determine the rate of islet cell autoimmunity and risk for T1D development in these patients.

To the best of our knowledge, our study was the first to evaluate islet autoimmunity and glucose metabolism in family members of AITD patients with islet AAb, indicating they are at risk for developing T1D, but this observation must be verified in larger studies.

Abbreviations

AAb Autoantibody

AITD Autoimmune thyroid disease

APS3v Autoimmune polyglandular syndrome type 3

AT Autoimmune thyroiditis

ELISA Enzyme-linked immunosorbent assay

GAD Glutamic acid decarboxylase autoantibody

GD Graves' Disease

IA2 Tyrosine-phosphatase autoantibody

IAA Insulin autoantibody

ICA Islet cell cytoplasmic autoantibody

JDF Juvenile Diabetes Foundation

OGTT Oral glucose tolerance test

T1D Diabetes mellitus type 1

Tg Thyroglobulin

TPO Thyroid peroxidase

Declarations

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Conflict of interest

The authors declare no competing interests.

Data availability

All data and materials support published claims and complied with field standards

Code availability

N/A

Authors' contributions

The final version of the manuscript has been read and approved by all the authors.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University of Zagreb, School of Medicine and Ethics Comittee of University Hospital Center Zagreb

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent for publication

Patients signed informed consent regarding publishing their data

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Tables

Table 1. The frequency (and percentage) of islet autoimmunity in 161 patients with autoimmune thyroid disease compared to 155 control subjects, as well as results of chi square and p value (<0.05) between the two groups, are presented

Patients	*Patients with islet autoimmunity (%)*	Chi square / p-value
AITD (n=161)	17 (10.6%)	**9.91 /0.002**
		6.37 / 0.01
AT (n=127)	15 (11.8%)	**11.39 / 0.0007**
		7.29/ 0.013
GD (n=34)	2 (5.9%)	1.68 / 0.19
Control group (n=155)	3 (1.9%)	

AITD - autoimmune thyroid disease; AT- autoimmune thyroiditis; GD – Graves' disease

Table 2. The frequency of islet autoantibodies in 161 patients with autoimmune thyroid disease (AITD) and 155 control patients. Data are presented as number of observations and percentage. Statistical significance for Chi square or Fisher between the two groups are presented.

Patients	*Islet AAb n (%)*			*One islet AAb (n)*			*Two islet AAb (n)*			*Three islet AAb (n)*
	ICA	*GAD*	*IA2*	*ICA*	*GAD*	*IA2*	*ICA+ GAD*	*ICA + IA2*	*GAD + IA2*	*ICA + GAD + IA2*
AITD (n=161)	9 (5.6%)	10 (6.2%)	8 (5.0%)	3	5	3	1	1	1	3
Controls (n=155)	2 (1.3%)	0 (0%)	1 (0.6%)	2	0	1	0	0	0	0
P (Chi square or Fisher's exact*)	0.04	0.002*	0.02							

AAb – autoantibodies; ICA -islets cell cytoplasmic autoantibody; GAD - glutamic acid decarboxylase autoantibody; IA2 - tyrosine-phosphatase autoantibody; AITD – autoimmune thyroid disease

* Statistical significance for Fisher's exact test

Table 3. Clinical characteristics, levels of thyroid and islet autoantibodies and evaluation of glucose metabolism in 17 patients with autoimmune thyroid disease and islet autoimmunity and 3 patients with islet autoimmunity in control group.

Patient #	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*
Dg	**AT**	**AT**	**GD**	**AT**	**AT**	**AT**	AT	AT	AT	AT	AT	AT	AT	AT	GD	AT
Age et AITD dg(yr)	**11.8**	**10.1**	**6.5**	**8.0**	**11.4**	**8.1**	13.5	11.3	19.0	11.5	12.3	7.8	13.2	12.2	15.4	13.7
Sex	**F**	**F**	**F**	**F**	**F**	**M**	F	F	F	F	F	F	F	F	F	M
TPO (U/mL)	**>2000**	**>2000**	**>2000**	**40**	**>2000**	**>2000**	269	>2000	159	>2000	1191	115	>2000	96	279	46
Tg (U/mL)	**44.2**	**0**	**71.1**	**140**	**25**	**584**	755	147	0	0	28.2	0	177.2	155	27	0
Age at evalation	**12.9**	**10.8**	**8.0***	**9.5**	**11.4**	**11.2**	16.8	14.3	20.4	14.2	16.0	11.3	14.9	14.2	16.0	16.6
ICA (JDF)	**330**	**290**	**370**	**285**	**65**	**50**	45	<5	<5	50	25	65	95	<5	60	25
GAD (U/mL) (IU/mL)	**676**	**859**	**2509**	**0**	**68.6**	**149**	0	22.7	17.7	23.7	0	0	0	27.4	0	0
IA2 (U/mL)	**258.6**	**136.1**	**1882**	**1245**	**226**	**0**	16.7	0	0	0	22.3	0	0	0	0	21.0
Age at T1D dg (yr)	**12.9**	**12.6**	**10.3**	**12.9**	**14.8**	**13.3**										
AgeAge at T1D dg(yr)																
AgeAge at T1D dg (yr)																
*HbA1c % (mmol/mol)***	**9.8 (84)**	**8.3 (67)**	**6.8 (51)**	**8.5 (69)**	**7.3 (56)**	**7.8 (62)**	5.5 (37)	5.2 (33)	5.0 (31)	5.2 (33)	5.3 (34)	4.7 (28)	4.8 (29)	5.2 (33)	5.3 (34)	5.0 (31)

Dg – diagnosis; yr – year; AT - autoimmune thyroiditis; GD – Graves disease; AITD – autoimmune thyroid disease, M – male; F – female; TPO - autoantibodies against thyroid peroxidase; Tg - autoantibodies against thyroglobulin; ICA – islet cell cytoplasmic autoantibody, GAD - glutamic acid decarboxylase autoantibody; IA2 - tyrosine-phosphatase autoantibody; NT – not tested

Bold - patients who developed T1D during the investigation period; * impaired glucose tolerance at the time of evaluation; **HbA1c %(mmol/mol) at the time of T1D diagnosis or the last HbA1c measured in patients with normal glucose metabolism

Table 4. Distribution of islet autoantibodies in 20 family members of 10 patients with autoimmune thyroid disease and islet autoimmunity.

Patient nos. (according to Table 3)	*Family member*	*Age at evaluation (years)*	*Islet AAb*		
			ICA (JDF)	*GAD (U/mL)*	*IA2 (U/mL)*
1	**1. a.mother***	**45**	**350**	**2890**	**1061**
	1.b.father	55	7	0	0
2	2.a.mother	36	10	0	0
	2.b.father **	**42**	**180**	**546**	**0**
	2.c.sister	5.8	0	9	0
4	4.a.mother	34	10	0	0
	4.b.father	39	105	2109	0
5	5.a.mother	47	0	0	0
	5.b.father	42	0	0	0
6	6.a.mother	45	0	0	0
	6.b.father	46	0	0	0
7	7.a.mother	46	10	0	0
	7.bfather	51	0	0	0
8	8.mother	42	0	0	0
	8.b.father	64	10	0	0
11	11.a.mother	45	15	0	24.5
	11.b.father	45	10	1871	0
12	12.a.mother	37	0	0	0
13	13.b.mother	45	0	0	0
	13.c.sister	17	0	0	0

AAb – autoantiboides; ICA -islets cell cytoplasmic autoantibody; GAD - glutamic acid decarboxylase autoantibody; IA2 - tyrosine-phosphatase autoantibody;

* Mother with previously diagnosed T1D; ** Father diagnosed with T1D at the age of 46 years