Clinical signs of type 1 diabetes are associated with type 2 diabetes marker transcription factor 7-like 2 polymorphism

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ABSTRACT

Aims/Introduction: We aimed to assess the distribution of transcription factor 7-like 2 gene *TCF7L2* (rs7903146) polymorphism and to find possible associations between *TCF7L2* and the characteristics of type 1 diabetes.

Materials and Methods: We studied 190 newly diagnosed type 1 diabetes patients (median age 12.7 years, range 2.0–72.5) and 246 controls (median age 23.8 years, range 1.4–81.5) for *TCF7L2* single nucleotide polymorphism. We determined anti-islet autoantibodies, random C-peptide levels, diabetes associated HLA DR/DQ haplotypes and genotypes in all patients.

Results: There were no differences in the distribution of *TCF7L2* single nucleotide polymorphism between patients and controls. However, patients with in type 1 diabetes, after adjusting for age and sex, subjects carrying *C* allele were at risk for a C-peptide level lower than 0.5 nmol/L (OR 5.65 [95% CI: 1.14–27.92]) and for zinc transporter 8 autoantibody positivity (5.22 [1.34–20.24]). Participants without *T* allele were associated with a higher level of islet antigen-2 autoantibodies (3.51 [1.49–8.27]) and zinc transporter 8 autoantibodies (2.39 [1.14–4.99]).

Conclusions: The connection of *TCF7L2* polymorphism with zinc transporter 8 and islet antigen-2 autoantibodies and C-peptide levels in patients supports the viewpoint that *TCF7L2* is associated with the clinical signs and autoimmune characteristics of type 1 diabetes. The mechanisms of the interaction between the *TCF7L2* risk genotype and anti-islet autoantibodies need to be studied further.

INTRODUCTION

Diabetes mellitus is a disease that is categorized according to the different mechanisms of pathogenesis, but shares a common ground with them: increased blood glucose levels, or hyperglycemia[1]. The type characterized by autoimmune destruction of beta-cells, is referred to as type 1 diabetes. In contrast, type 2 diabetes derives from many different pathophysiological processes including lower ability of insulin to stimulate glucose uptake, lower ability of glucose to stimulate and suppress its own uptake, and suppression of insulin

action[1,2]. Regardless of the mechanism of pathogenesis, both type 1 diabetes and type 2 diabetes share similar signs and symptoms, while their onset and development are different[2].

The mechanisms underlying type 1 diabetes related beta-cell destruction have been studied thoroughly and can be examined under environmental and genetic factors[3–5]. Concerning genetic triggers, associations between human leukocyte antigen (HLA) and type 1 diabetes were studied nearly half a century ago[6]. The results indicated that specific HLA regions were spotted more often in insulin dependent diabetes[6]. Overall, it was shown that HLA class II DQ-DR loci on chromosome 6 were of great importance and haplotypes DR3-DQ2 and DR4-DQ8 had a synergistic effect in pathogenesis[7–9].

According to a genome-wide association study (GWAS), one single nucleotide polymorphism (SNP) of gene transcription factor 7-like 2 (*TCF7L2*) was the most significantly associated signal in type 2 diabetes patient groups[10,11]. The *TCF7L2* is a transcription factor that forms part of the lymphopoiesis influencing the Wnt signaling pathway[12]. It also plays an important role in the development of the intestinal epithelium, as *TCF7L2* knock-out mice cannot survive more than 1 day according to previous studies[13]. Supporting this, the loss of *TCF7L2* impairs the morphology of cells as shown in another recent study[14]. Yet the mechanism is still being investigated. The *TCF7L2* regulates the expression of proglucagon gene (*glu*), supporting the production of glucagon and glucagon like peptide-1 (GLP-1)[2,15]. The opposing glucagon, GLP-1, stimulates insulin production, and lowers blood sugar level[2,15]. Recent studies have shown that even though *TCF7L2* is a transcription factor known to be associated with type 2 diabetes, it can also have unexpected effects on the pathogenesis of type 1 diabetes. For example, it was found that *TCF7L2* rs7903146 *C*-to-*T* polymorphism (*T*-risk allele) decelerates single to multiple autoantibody progression in type 1 diabetes patients with low-risk HLA loci, demonstrating a protective effect[16].

Proceeding from previous knowledge and the above research, we aimed to assess the prevalence of the risk genotype in the Estonian population and its possible association with type 1 diabetes markers, including clinical and baseline characteristics and autoantibody presence, which influence the mechanism of pathogenesis of type 1 diabetes.

MATERIALS AND METHODS

Study population

This study included 190 recent-onset type 1 diabetes patients (2.0–72.5 years old, median age 12.7 years, 105 males and 85 females). A total of 246 unrelated participants without anti-islet autoantibodies were included in the study as controls (1.4–81.5 years old, median age 23.8 years, 90 males and 156 females) (Table 1). The study was approved by the Research Ethics Committee of the University of Tartu (protocols 163/T-6 from 24.09.2007 and 275/M/15 from 20.11.2017). All participants or their parents (or guardians, if needed) signed a written informed consent form before participation.

Patients with type 1 diabetes were recruited from the Children's Clinic of Tartu University Hospital, from Tallinn Children's Hospital and from the Internal Medicine Clinic of Tartu University Hospital between October 2004 and March 2020. Patients with type 1 diabetes were diagnosed by using the internationally accepted diagnosis criteria[1]. Their peripheral blood samples were obtained up to 30 days after the diagnosis (1–26 days). The random C-peptide levels of the patients were 0.01–2.78 nmol/L (median = 0.23 nmol/L, cut-off value = 0.5 nmol/L were used[17]). C-peptide levels were lower than 0.5 nmol/L in more than half of the patients (87.7%). Two participants in the type 1 diabetes group were young children with C-peptide levels higher than the reference range. For

further calculations, they were added to the >0.5 nmol/L C-peptide group. More details about the patient group and the control group are presented in Table 1.

The control group for this study consisted of non-diabetic children visiting the Surgery Clinic of Tartu University Hospital and volunteers. All controls had normal HbA1c and/or normal glucose levels and had no anti-islet autoantibodies. For the study, controls and patients were recruited synchronously.

Variables

Information on the baseline characteristics including age, sex, body mass index (BMI), family history of type 1 diabetes, and concomitant autoimmune diseases were obtained from patients and controls at the time of recruitment. Clinical variables, including duration of symptoms, random C-peptide levels, and the presence of ketonuria and ketoacidosis, were obtained for the patient group.

Detection of antibodies

Autoantibodies against glutamic acid decarboxylase (GADA), islet antigen-2 (IA2A), and zinc transporter 8 (ZnT8A) were detected in patients with type 1 diabetes and in controls using commercial ELISA kits in compliance with the manufacturer's instructions (RSR Ltd, Cardiff, UK). The cut-off values indicating antibody positivity were ≥5 U/mL for GADA and ≥15 U/mL for ZnT8A. The cut-off values indicating IA2A positivity were ≥15 U/mL (tests up to May 2015) or ≥7.5 U/mL (tests after May 2015). All tests are regularly validated as external quality assurance by the Islet Autoantibody Standardization Program[18]. For statistical analysis, the results for autoantibodies were divided into two groups: (1) lower level, i.e. values lower than median and (2) higher level, i.e. values equal to or higher than median (Table 1).

Genotyping

For *HLA-DR/DQ* genotyping, PCR-based lanthanide labeled oligonucleotide hybridization with time resolved fluorometry was used as portrayed in the literature[19,20]. Comparison groups were created regarding the following combinations of *HLA-DRB1-DQA1-DQB1* haplotypes: (1) *DR3/DR4*, (2) *DR4/x*, (3) *DR3/x*, and (4) *x/x*, where the haplotypes that were not *DR3* or *DR4* were denoted by *x*. Grouping was carried out according to Ilonen *et al.*[9]

DNA was extracted from peripheral blood leukocytes using the salting out method. The *TCF7L2* rs7903146 were genotyped using the TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA, USA) on the ABI 7000 instrument (Applied Biosystems). The SNPs were genotyped according to the manufacturer's protocol (Applied Biosystems). All investigated polymorphisms were in the Hardy–Weinberg equilibrium ($P > 0.05$).

Statistical analysis

Type 1 diabetes patients and controls were first evaluated using descriptive statistics. Continuous variables were expressed as

Table 1 | Summarized characteristics of the study participants

Characteristic	Type 1 diabetes patients (*n* = 190)	Controls (*n* = 246)
Male, % (*n*)	55.3 (105/190)	36.6 (90/246)
Age (years), median (IQR)	12.7 (8.7–26.3)	23.8 (16.6–32.2)
Duration of symptoms (days), median (IQR)	30 (14–60)	–
BMI, % (*n*)		
Underweight	19.1 (18/94)	–
Normal weight	50.0 (47/94)	
Overweight	20.2 (19/94)	
Obesity	10.6 (10/94)	
Ketonuria, % (*n*)		
Yes	73.0 (130/178)	–
No	27.0 (48/178)	
Ketoacidosis, % (*n*)		
Yes	35.5 (66/186)	–
No	64.5 (120/186)	
C-peptide (nmol/L), median (IQR)	0.23 (0.13–0.36)	–
C-peptide, % (*n*)		
<0.5 nmol/L	87.7 (136/155)	–
≥0.5 nmol/L	12.3 (19/155)	
AAB positive persons, % (*n*)		
GADA	79.5 (151/190)	0 (0/246)
IA2A	57.9 (110/190)	0 (0/246)
ZnT8A	68.4 (130/190)	0 (0/246)
AAB levels (U/mL), median (IQR)		
GADA	150 (35.5–1,900)	
IA2A	380 (60–4,000)[†]	
ZnT8A	370 (98.3–950)	
AAB count, % (*n*)		
0	8.4 (16/190)	100 (246/246)
1	17.4 (33/190)	0 (0/246)
2	34.2 (65/190)	0 (0/246)
3	40 (76/190)	0 (0/246)
HLA risk, % (*n*)		
DR3/DR4	25.8 (49/190)	0.4 (1/246)
DR4/x	26.8 (51/190)	18.3 (45/246)
DR3/x	24.7 (47/190)	18.3 (45/246)
x/x	22.6 (43/190)	63.0 (155/246)

AAB, (anti-islet) autoantibodies; BMI, body mass index; GADA, autoantibodies against glutamic acid decarboxylase; HLA, human leukocyte antigen; IA2A, autoantibodies against islet antigen-2; IQR, interquartile range; *n*, count; ZnT8A, autoantibodies against zinc transporter 8.
[†]Results are presented for values detected after May 2015. Median values detected before May 2015 were 320 (IQR 92.5–1750).

median and interquartile range (*IQR*) and non-parametric data were provided as count and percentage. For comparison of categorical variables, the χ^2 test or Fisher's exact test was employed. For continuous variables, the Mann–Whitney *U*-test or the Kruskal–Wallis test was used for comparison. Logistic regression models adjusted for age and gender were used to evaluate the influence of *TCF7L2* on the outcome of interest. A

linear regression model with log-transformation, adjusted for age and gender, was used for variables with a normal distribution. The R: A Language and Environment for Statistical Computing (version 3.6.1; R core Team, Vienna, Austria) was used for statistical analyses. Values of *P* <0.05 were evaluated as statistically significant. The Hardy–Weinberg equilibrium of SNPs was verified with the χ^2 test. There was no significant variation in the studied populations and their equilibrium was not significantly different.

RESULTS

Distribution of genotypes in the study populations

The results of genotyping *TCF7L2* rs7903146 revealed that 11 of the 190 (5.8%) patients with type 1 diabetes and 11 of the 246 (4.5%) controls were carrying the type 2 diabetes risk genotype *TT*. Among the 190 patients with type 1 diabetes, 120 had the *CC* genotype (63.2%) and 59 had the *CT* genotype (31.1%). The distribution of the *TCF7L2* genotypes and alleles did not reveal any significant difference between patients with type 1 diabetes and the controls. Additional information is presented in Table 2.

Associations with baseline characteristics of controls

Initially we evaluated the *TCF7L2* rs7903146 genotypes and alleles in controls against the HLA risk genotypes, age and sex, however, we did not establish any significant association between them (see Tables S1 and S2).

Associations with baseline clinical characteristics of patients with type 1 diabetes

Further, the effects of *TCF7L2* rs7903146 polymorphism were investigated in patients with type 1 diabetes. Additional information is presented in Table 3 and Table S3. Median C-peptide levels were similar between the different genotypes (Figure S1). However, we found a significant association of genotypes with C-peptide levels <0.5 nmol/L and ≥0.5 nmol/L (*P* = 0.0437; Table 3). A similar tendency was also found between these C-peptide levels and genotype groups (*P* = 0.0591; Table S3). We did not establish significant association of genotype or genotype group with onset variables (ketoacidosis, ketonuria, or duration of symptoms), HLA risk genotypes or any other disease characteristics (Table 3 and Table S3).

As a next step, we attempted to find out if *TCF7L2*, age and sex have any effect on a C-peptide level lower than <0.5 nmol/L in the logistic regression model. Logistic regression analysis, adjusted for age and sex, revealed that the *CC/CT* group was the risk factor vs the *TT* group (OR 5.65, *P* = 0.0336) for having a C-peptide level of <0.5 nmol/L. Older age (OR 0.96, *P* = 0.0122) was a protective factor for a C-peptide level of <0.5 nmol/L (Table 4). Further, we also examined the genotypes and found that the *CC* genotype was a risk factor for having a C-peptide level of <0.5 nmol/L vs the *TT* genotype

Table 2 | Distributions of the *TCF7L2* (rs7903146) genotypes and alleles in the study population

TCF7L2	All (*n* = 436)	Type 1 diabetes patients (*n* = 190)	Controls (*n* = 246)	*P*-value
Genotype, % (*n*)				
CC	60.1 (262/436)	63.2 (120/190)	57.7 (142/246)	0.3170
CT	34.9 (152/436)	31.1 (59/190)	37.8 (93/246)	
TT	5.0 (22/436)	5.8 (11/190)	4.5 (11/246)	
CT + TT	40.3 (174/436)	36.8 (70/190)	42.3 (104/246)	0.2936
Allele, % (*n*)				
C	77.5 (676/872)	78.7 (299/380)	76.6 (377/492)	0.5221
T	22.5 (196/872)	21.3 (81/380)	23.4 (115/492)	

(OR 7.04, *P* = 0.0214) and older age (OR 0.96, *P* = 0.0122; Table S4).

Associations with autoantibodies in patients with type 1 diabetes

A significant association was found between the *TCF7L2* genotypes and ZnT8A positivity (*P* = 0.0375; Table 3). Also, we noted a significant association between the genotype groups (CT/TT and CC) and higher and lower levels of ZnT8A (*P* = 0.0484) (data not shown). Regarding the genotype group, there was a significant association with ZnT8A positivity (*P* = 0.0384; Table S3). Although there was no significant association between IA2A positivity and *TCF7L2* genotypes, we found a statistically significant association of *TCF7L2* genotypes and genotype groups with lower and higher levels of IA2A (*P* = 0.0011 and *P* = 0.0129, respectively). We also examined the distribution of *TCF7L2* genotypes (also with genotype groups) between patients with type 1 diabetes with single autoantibody positivity vs ≥2 autoantibody positivity and autoantibody negativity vs autoantibody positivity, but we failed to find any significant differences in this regard (Table 3 and Table S3).

As a next step, we attempted to find out whether *TCF7L2*, age, and sex have any effect on antibodies in the logistic regression model. First, we considered risk factors for ZnT8A positivity. For patients with C allele (CC/CT group), the likelihood of developing ZnT8A positivity was 5.22 times higher than for patients without C allele (*P* = 0.0170; Table 5). In the model of genotypes, the CC and CT genotypes were both significant risk factors for ZnT8A positivity (OR 4.69, *P* = 0.0277 and OR 6.65, *P* = 0.0115, respectively; Table S5). Older age was a protective factor for ZnT8A positivity in both models (OR 0.93, *P* < 0.0001 and OR 0.93, *P* < 0.0001, respectively) after adjusting for age and sex. Next, logistic regression analysis was performed for higher levels of ZnT8A. The CC group was at risk of having higher levels ZnT8A compared with the CT/TT group (OR 2.39, *P* = 0.0204) after age and sex adjustment (Table 6). There was no significant association with higher level of ZnT8A and genotypes in logistic regression analysis.

We continued with the assessment of risk factors for a higher level of IA2A, using logistic regression analysis. After adjustment for age and sex, the presence of the CC group was found to be a risk factor against having a higher level of IA2A compared with the CT/TT group (OR 3.51, *P* = 0.0042; Table 7).

DISCUSSION

In this study, our aim was to explore the prevalence of a type 2 diabetes marker, *TCF7L2*, in patients with type 1 diabetes in the Estonian population and to find out what other parameters could be linked with *TCF7L2* rs7903146 polymorphism in these patients.

According to the inclusion criteria for our study group, the patients' blood samples were taken within 30 days of diagnosis of type 1 diabetes. Using these criteria, we aimed to investigate early changes that occur prior to the diagnosis and attempted to exclude long-term compensatory mechanisms for changes that occur due to the onset of insulin deficiency.

First, we did not find any association between the *TCF7L2* rs7903146 TT genotype and the presence of type 1 diabetes (Table 2). This finding supports previous results obtained for *TCF7L2* and type 1 diabetes, showing that the presence of the *TCF7L2* rs7903146 TT genotype in type 1 diabetes patients is not related to autoimmunity mediated destruction in the beta-cells of the pancreas[21–26].

However, we found that carrying the *TCF7L2* risk genotype has an effect on C-peptide levels in patients diagnosed with type 1 diabetes. We showed that the frequency of patients with type 1 diabetes with random C-peptide levels of <0.5 nmol/L were higher in carriers of the *TCF7L2* rs7903146 C allele. A similar tendency was seen in the case of the genotypes. As C-peptide levels indicate insulin production capacity and islet dysfunction, we can suggest that carrying the *TCF7L2* rs7903146 T allele may result in preservation of beta-cell function. Bakhtadze *et al.* have reported that the *TCF7L2* rs7903146 CT/TT genotypes are associated with GADA negative diabetes in younger patients, but not in middle-aged patients, making the risk allele an important indicator of milder islet autoimmunity[27]. However, that study included both autoimmune and non-

Table 3 | Associations between the *TCF7L2* genotypes and baseline clinical characteristics of type 1 diabetes patients

Characteristic	*CC* genotype (*n* = 120)	*CT* genotype (*n* = 59)	*TT* genotype (*n* = 11)	*P*-value
Male, % (*n*)	54.2 (65/120)	57.6 (34/59)	54.5 (6/11)	0.9392
Age (years), median (IQR)	13.9 (9.4–25.9)	11.5 (6.9–25.9)	12.2 (10.6–29.7)	0.2659
Duration of symptoms (days), median (IQR)	30 (14–45)	30 (14–60)	14 (11–53)	0.6168
BMI, % (*n*)				
Underweight	19.4 (12/62)	8.5 (5/27)	9.1 (1/5)	0.9757
Normal weight	50.0 (31/62)	23.7 (14/27)	18.2 (2/5)	
Overweight	16.1 (12/62)	10.2 (6/27)	9.1 (1/5)	
Obesity	11.3 (7/62)	3.4 (2/27)	9.1 (1/5)	
Ketonuria, % (*n*)	70.8 (80/113)	75.0 (42/56)	88.9 (8/9)	0.5405
Ketoacidosis, % (*n*)	36.1 (43/119)	31.0 (18/58)	55.6 (5/9)	0.3793
C-peptide (nmol/L), median (IQR)	0.23 (0.13–0.33)	0.21 (0.15–0.41)	0.23 (0.11–0.65)	0.7404
C-peptide, % (*n*)				
<0.5 nmol/L	91.0 (91/100)	85.1 (40/47)	62.5 (5/8)	**0.0437**
≥0.5 nmol/L	9.0 (9/100)	14.9 (7/47)	37.5 (3/8)	
GADA positivity, % (*n*)	83.3 (100/120)	71.2 (42/59)	81.8 (9/11)	0.1767
GADA (U/mL), median (IQR)	135 (34–1825)	150 (36–2000)	160 (43–310)	0.9254
GADA level, % (*n*)				
Lower level	51.0 (51/100)	50.0 (21/42)	44.4 (4/9)	0.9629
Higher level	49.0 (49/100)	50.0 (21/42)	55.6 (5/9)	
IA2A positivity, % (*n*)	58.3 (70/120)	55.9 (33/59)	63.6 (7/11)	0.9107
IA2A level, % (*n*)				
Lower level	40.0 (28/70)	63.6 (21/33)	100.0 (7/7)	**0.0011**
Higher level	60.0 (42/70)	36.4 (12/33)	0.0 (0/7)	
ZnT8A positivity, % (*n*)	67.5 (81/120)	76.3 (45/59)	36.4 (4/11)	**0.0375**
ZnT8A (U/mL), median (IQR)	450 (112–1,175)	225 (68–775)	175 (121–267)	0.1897
ZnT8A level, % (*n*)				
Lower level	42.7 (35/82)	60.9 (28/46)	75.0 (3/4)	0.0915
Higher level	57.3 (47/82)	39.1 (18/46)	25.0 (1/4)	
AAB count, % (*n*)				
Single AAB positive	18.0 (20/111)	18.9 (10/53)	30.0 (3/10)	0.6071
Multiple AAB positive	82.0 (91/111)	81.1 (43/53)	70.0 (7/10)	
AAB positivity, % (*n*)				
AAB negative	7.5 (9/120)	10.2 (6/59)	9.1 (1/11)	0.6772
AAB positive	92.5 (111/120)	89.8 (53/59)	90.9 (10/11)	
HLA risk group, % (*n*)				
DR3/DR4	23.3 (28/120)	32.2 (19/59)	18.2 (2/11)	0.5984
DR4/x	26.7 (32/120)	25.4 (15/59)	36.4 (4/11)	
DR3/x	23.3 (28/120)	27.1 (16/59)	27.3 (3/11)	
x/x	26.7 (32/120)	15.3 (9/59)	18.2 (2/11)	

P-values in bold are statistically significant. AAB, (anti-islet) autoantibodies; BMI, body mass index; GADA, autoantibodies against glutamic acid decarboxylase; HLA, human leukocyte antigen; IA2A, autoantibodies against islet antigen-2; IQR, interquartile range; *n*, count; ZnT8A, autoantibodies against zinc transporter 8.

autoimmune diabetic patients and did not clearly differentiate between the possible effects of the risk genotypes on different types of diabetes conditions[27]. Nor did our study find a significant association between *TCF7L2* and GADA positivity. Furthermore, Redondo *et al.* reported in several studies that *TCF7L2* risk variants were associated with higher C-peptide levels and single autoantibody positivity, which is consistent with the findings of our study[16,28].

Also, we found an association between the presence of ZnT8A and *TCF7L2* genotypes. Apart from patients having the

TCF7L2 rs7903146 *CC* and *CT* genotypes, patients carrying the *TCF7L2* rs7903146 risk genotype *TT* had less ZnT8A positivity. Therefore, we believe that the association between ZnT8A and *TCF7L2* deserves special attention. ZnT8 is a self-antigen, present in the pancreatic islet cells, which is related to insulin secretion. The more insulin is secreted, the more ZnT8 is expressed on the surface of pancreatic islet cell[29]. According to Zhou *et al.*[30] the *TCF7L2*-regulated transcription network has been described to be connected with *SLC30A8* gene encoding ZnT8. Silencing of *TCF7L2* gene expression in donor human

Table 4 | Logistic regression analysis evaluating the association of the *TCF7L2* genotype groups, age, and sex with a C-peptide level lower than 0.5 nmol/L vs higher than 0.5 nmol/L at the time of the diagnosis of type 1 diabetes

Characteristic	Unadjusted OR (95% CI)	*P*-value	Adjusted OR[†] (95% CI)	*P*-value
Genotype group				
TT	1[‡]		1[‡]	
CC + CT	4.91 (1.07–22.52)	**0.0405**	5.65 (1.14–27.92)	**0.0336**
Age (years)	0.97 (0.94–0.99)	**0.0153**	0.96 (0.94–0.99)	**0.0122**
Sex				
Male	1[‡]		1[‡]	
Female	1.44 (0.53–3.87)	0.4741	1.34 (0.48–3.74)	0.5821

P-values in bold are statistically significant. CI, confidence interval; OR, odds ratio. [†]Adjusted for age and sex. [‡]Reference.

Table 5 | Logistic regression analysis evaluating the association of the *TCF7L2* genotype groups, age, and sex with ZnT8A positivity at the time of the diagnosis of type 1 diabetes

Characteristic	Unadjusted OR (95% CI)	*P*-value	Adjusted OR[†] (95% CI)	*P*-value
Genotype group				
TT	1[‡]		1[‡]	
CC + CT	4.16 (1.17–14.81)	**0.0278**	5.22 (1.34–20.24)	**0.0170**
Age (years)	0.93 (0.91–0.96)	**<0.0001**	0.93 (0.91–0.96)	**<0.0001**
Sex				
Female	1[‡]		1[‡]	
Male	1.02 (0.55–1.88)	0.9605	1.29 (0.64–2.61)	0.4893

P-values in bold are statistically significant. CI, confidence interval; OR, odds ratio. [†]Adjusted for age and sex. [‡]Reference.

Table 6 | Logistic regression analysis evaluating the association of the *TCF7L2* genotype groups, age and sex with higher level of ZnT8A vs lower level of ZnT8A at the time of the diagnosis of type 1 diabetes

Characteristic	Unadjusted OR (95% CI)	*P*-value	Adjusted OR[†] (95% CI)	*P*-value
Genotype group				
TT + CT	1[‡]		1[‡]	
CC	2.19 (1.07–4.50)	**0.0326**	2.39 (1.14–4.99)	**0.0204**
Age (years)	0.98 (0.96–1.02)	0.3180	0.98 (0.95–1.01)	0.1855
Sex				
Male	1[‡]		1[‡]	
Female	1.06 (0.54–2.11)	0.8610	1.04 (0.51–2.12)	0.9147

P-values in bold are statistically significant. CI, confidence interval; OR, odds ratio. [†]Adjusted for age and sex. [‡]Reference.

Table 7 | Logistic regression analysis evaluating the association of the *TCF7L2* genotype groups, age, and sex with higher level of IA2A vs lower level of IA2A at the time of the diagnosis of type 1 diabetes

Characteristic	Unadjusted OR (95% CI)	*P*-value	Adjusted OR[†] (95% CI)	*P*-value
Genotype group				
TT + CT	1[‡]		1[‡]	
CC	3.50 (1.53–8.01)	**0.0326**	3.51 (1.49–8.27)	**0.0042**
Age (years)	0.99 (0.96–1.02)	0.5191	0.98 (0.95–1.01)	0.2575
Sex				
Female	1[‡]		1[‡]	
Male	2.28 (1.06–4.92)	**0.0350**	2.11 (0.94–4.73)	0.0691

P-values in bold are statistically significant. CI, confidence interval; OR, odds ratio. [†]Adjusted for age and sex. [‡]Reference.

pancreatic islet cells led to a weaker expression of ZnT8[30]. Moreover, Dwivedi *et al.*[31] reported recently that the loss of function of ZnT8-coding gene *SLC30A8* is associated with better insulin secretion and protection against type 2 diabetes. It is worth mentioning that the above study used the gene silencing method, but the effects of SNPs of *TCF7L2* on ZnT8 expression were not confirmed. Redondo *et al.*[28] reported previously in a cohort of children that a higher frequency of the *TT* genotype was linked with single autoantibody positivity and children with single autoantibody positivity were less likely to express ZnT8A. Confirming these findings, we found that carrying the type 2 diabetes risk *TCF7L2* genotype (*TT*) was associated with ZnT8A negativity. Also subjects with *T* allele (*CT/TT*) had a lower level of ZnT8A, which may yield a potential protective pathogenetic mechanism in patients with type 1 diabetes, leading to milder islet autoimmunity. It is quite difficult to explain this association, since *TCF7L2* may play an important role in different cellular signaling pathways while some of them may have an indirect effect on diabetes. However, to our knowledge, this is the first study that shows the presence of an association contributing to the above mentioned milder islet autoimmunity with the *TCF7L2* rs7903146 risk genotype *TT*. Therefore, these associations need further research.

Additionally, another important association was found between the IA2A and *TCF7L2* genotype groups. IA2 autoantigen is located at the insulin secretory granule membrane. It is thought that during granule exocytosis, the cytosolic fragment of IA2 is cleaved to have effects on the nucleus for transcription of granule genes (insulin, IA2)[32]. Its role in the insulin secretory granule has important consequences in the pathogenesis of type 1 diabetes. For instance, the presence of IA2A in first degree relatives can lead to a 50% cumulative risk for the development of type 1 diabetes within 10 years[33]. Moreover, it was previously shown that when IA2A or IAA is present as a single autoantibody in overweight/obese patients, the likelihood of progression to multiple autoantibody positivity in 5 years was 83% greater in *TCF7L2* risk *T* allele carriers[16]. Redondo *et al.* have shown in several studies that associations of the *TCF7L2* risk variant is more frequently established in distinct type 1 diabetes patient groups, such as individuals with fewer islet autoimmunity markers[28,34,35]. Supporting these findings, our study demonstrates that *T* allele (*CT/TT*) is associated with a lower titer of IA2A in type 1 diabetes patients, meaning that it can contribute to milder type 1 diabetes. Whether this is related to a change of the *TCF7L2* function in antibody producing cells, thymus or elsewhere requires further investigations. It has been found that *TCF7L2* plays an important role in different cells and tissues that are involved in the development of diabetes and changes in glucose metabolism[36].

Among the limitations of this study is the relatively small sample size, which included 190 type 1 diabetes patients and 246 controls. The non-match of the controls and the patients according to age and sex could have affected the findings. Additionally, data on fasting C-peptide levels of the study participants were not available and random C-peptide levels were used for analysis. The notable strengths of this study were the inclusion criteria and the detailed grouping of the study sample.

In terms of consistency with other studies, it is evident, as associations were found between type 2 diabetes associated gene *TCF7L2* and some type 1 diabetes markers, C-peptide levels, IA2A and ZnT8A, that research should be continued focusing on associations of *TCF7L2* at the beta cell level. We believe that the establishment of cellular mechanisms connecting C-peptide levels with autoantibodies and the *TCF7L2* rs7903146 *TT* genotype would help the scientific community to better understand the nature of type 1 diabetes. In addition, we emphasize the prominent pathogenetic role of *TCF7L2* in a wide range of other diseases, as pointed out also in a thorough recent overview by del Bosque-Plata *et al.*[36].

DISCLOSURE

All authors declare no conflicts of interest.

This study was conducted in accordance with the Declaration of Helsinki.

Approval of the research protocol: The study was approved by the Research Ethics Committee of the University of Tartu (protocols 163/T-6 from 24.09.2007 and 275/M/15 from 20.11.2017).

Informed consent: Written informed consent was obtained from all participants and controls (in the case of children from their parent).

Registry and the registration no. of the study/trial: N/A.
Animal studies: N/A.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 | Median C-peptide level with the interquartile range (IQR) between the *TCF7L2* genotypes of patients with type 1 diabetes.

Table S1 | Associations between the *TCF7L2* genotypes and baseline clinical characteristics of the control group.

Table S2 | Associations between the *TCF7L2 CC/CT* group and *TT* group and baseline clinical characteristics of the control group.

Table S3 | Associations between the *TCF7L2 CC/CT* group and *TT* group and baseline clinical characteristics of patients with type 1 diabetes.

Table S4 | Logistic regression analysis evaluating the association of the *TCF7L2* genotypes, age, and sex with a C-peptide level lower than 0.5 nmol/L, vs higher than 0.5 nmol/L, at the time of diagnosis of type 1 diabetes.

Table S5 | Logistic regression analysis evaluating the association of the *TCF7L2* genotypes, age and sex with ZnT8A positivity at the time of diagnosis of type 1 diabetes.