Association of TCF7L2 variation with single islet autoantibody expression in children with type 1 diabetes

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ABSTRACT

Background: The transcription factor 7-like 2 (TCF7L2) gene has the strongest genetic association with type 2 diabetes. TCF7L2 also associates with latent autoimmune diabetes in adults, which often presents with a single islet autoantibody, but not with classical type 1 diabetes.

Methods: We aimed to test if TCF7L2 is associated with single islet autoantibody expression in pediatric type 1 diabetes. We studied 71 prospectively recruited children who had newly diagnosed type 1 diabetes and evidence of islet autoimmunity, that is, expressed ≥2 islet autoantibody to insulin, glutamic acid decarboxylase 65, islet cell autoantigen 512, or zinc transporter 8. TCF7L2 rs7903146 alleles were identified. Data at diagnosis were cross-sectionally analyzed.

Results: We found that 21.1% of the children with autoimmune type 1 diabetes expressed a single islet autoantibody. The distribution of TCF7L2 rs7903146 genotypes in children with a single autoantibody (n=15) was 40% CC, 26.7% CT and 33.3% TT, compared with children with ≥2 islet autoantibodies (50% CC, 42.9% CT and 7.1% TT, p=0.024). Furthermore, compared with children with ≥2 autoantibodies, single-autoantibody children had characteristics reflecting milder autoimmune destruction of β-cells. Restricting to lean children (body mass index<85th centile; n=36), 45.5% of those expressing a single autoantibody were rs7903146 TT homozygotes, compared with 0% of those with ≥2 autoantibodies (p<0.0001).

Conclusion: These results suggest that, in children with only mild islet autoimmunity, mechanisms associated with TCF7L2 genetic variation contribute to diabetogenesis, and this contribution is larger in the absence of obesity.

Key messages

- Expression of single islet autoantibody was found in about 20% of children with new onset type 1 diabetes and was associated with clinical characteristics of milder autoimmune destruction of beta-cells, compared to those of children expressing ≥2 autoantibodies.
- In children with type 1 diabetes and only mild islet autoimmunity, as reflected by expression of a single islet autoantibody, the TCF7L2 genetic variant that is associated with type 2 diabetes and LADA was more common than in children with type 1 diabetes and two or more positive islet autoantibodies.
- The association between the TCF7L2 genetic variant and single-autoantibody pediatric type 1 diabetes was stronger in the absence of obesity.
pharmacological agents, such as incretin enhancers and metformin, respectively.

Positivity for any of the islet autoantibodies is the most widely used parameter to define autoimmune type 1 diabetes. The number of circulating islet autoantibodies is a strong determinant of subsequent progression to type 1 diabetes in individuals without diabetes at risk and the general population, while expression of a single autoantibody confers only low risk of developing type 1 diabetes. Although studies on β-cell function after type 1 diabetes onset are lacking in children, particularly below age 7, adults with LADA, who have slowly progressive loss of β-cell function after the onset of diabetes, often express a single autoantibody, which further supports the notion that a single autoantibody expression is a marker of milder β-cell autoimmunity.

We hypothesized that patients who develop autoimmune type 1 diabetes during childhood despite only mild islet autoimmunity, as reflected by possessing a single positive islet autoantibody, are more likely to harbor the diabetogenic TCF7L2 rs7903146 TT genotype. Hence, the goal of this study was to compare, in a cohort of children with newly diagnosed autoimmune type 1 diabetes, the frequency of the rs7903146 TT genotype and clinical features among those with a single islet autoantibody and those with ≥2 positive islet autoantibodies.

**METHODS**

**Subjects**

We prospectively recruited 119 children who presented with newly diagnosed diabetes at Texas Children’s Hospital between October 2010 and October 2011. The study was approved by the Institutional Review Board of Baylor College of Medicine. Fasting C peptide concentrations were measured ≥3 weeks after diagnosis (median=7 weeks, SD=3) to avoid the potential effect on β-cell function of metabolic instability and diabetic ketoacidosis (DKA) that may accompany onset of disease. Insulin autoantibodies were measured within 10 days of diagnosis (prior to development of antibodies to exogenous insulin). Other islet autoantibodies—to glutamic acid decarboxylase 65 (GAD65), islet cell autoantigen 512 (ICA512/IA2) and zinc transporter 8 (ZnT8)—were measured at the 7-week (median, SD=3) visit. For this cross-sectional analysis, we included only children with evidence of autoimmune diabetes to prevent including cases of type 2 diabetes, which is known to associate with the genetic variant under study. We excluded those who did not have all four islet autoantibodies measured (n=27) and those who were negative for all four islet autoantibodies (n=21). The final sample comprised 71 patients.

**Data collection**

Demographic information included date of birth, date of diagnosis, sex, and race/ethnicity. Anthropometric information included weight and height measured at the first clinical visit (mean±SD=10.3 weeks±5.4) to avoid the effect of dehydration on weight at the time of diagnosis. Tanner pubertal staging was performed by a pediatric endocrinologist. Biochemical data included glucose, glycated hemoglobin (HbA1c), pH, bicarbonate, and β-hydroxybutyrate measured at diagnosis. Body mass index (BMI) was calculated in children older than 2 years based on height and weight and was categorized using sex-specific and age-specific percentiles by Centers for Disease Control and Prevention criteria. Obesity was defined as BMI at or above the 95th sex-specific and age-specific BMI centile, and overweight as BMI centile ≥85th to <95th. In children below age 2 years (n=11), BMI was considered a missing value because of the lack of standardized age-adjusted and gender-adjusted BMI data for ages 0–2 years. DKA was defined by venous blood pH<7.3 and bicarbonate <15 mEq/L.

**Laboratory methods**

GAD65, ICA512/IA2, and ZnT8 autoantibodies were measured by the radioligand binding assay as previously described. Samples were considered ICA512/IA2 autoantibody positive if binding exceeded that of the 98th centile for healthy controls (30 RU/mL). For ZnT8 autoantibodies, a cutoff was set at 15 RU/mL for autoantibodies for ZnT8Arg and 26 RU/mL for ZnT8Tsp based on the 98th centile observed in 50 healthy human control sera. Samples were considered ZnT8 autoantibody positive if binding to either ZnT8Arg or ZnT8Tsp was detected. Our laboratory participated in the Diabetes Autoantibody Standardization Program (DASP) workshop and the GAD65 autoantibody assay showed 86% sensitivity and 93% specificity, and the ICA512/IA2 autoantibody assay showed 66% sensitivity and 98% specificity. ZnT8 autoantibodies were not included in the workshop. Insulin autoantibodies were measured by the Quest Diagnostics Nichols Institute (San Juan Capistrano, California, USA) by radioimmunoassay (RIA) with clinical sensitivity and specificity of 50% and 99%, respectively (positivity=0.4 U/mL). Serum C peptide was measured by highly specific RIA (Human C Peptide RIA kit, Millipore Research Inc, St Louis, Missouri, USA).

**Genetic methods**

Genomic DNA was extracted from peripheral blood leukocytes by standard methods as previously described. Multiple displacement amplification (MDA) of genomic DNA, PCR, and direct DNA sequencing were performed as previously described. Heterozygous bases were called if the minor peak was ≥25% the height of the major peak. TaqMan assays: Approximately 10–100 ng MDA DNA was used as the template according to the manufacturer’s protocol. For the TCF7L2 rs7903146 allele, we designed primers and probe using the Life Technologies custom TaqMan assay website (5’-CTTCA AACCTAGGACAGCTTAT [forward primer], 5’-TGAA...
A base calls were con

the Hardy-Weinberg equilibrium. To compare proportions among groups, we

Statistical analyses were conducted with STATA V.10

sequence alignments.

were used to compare rank distributions of a continu-

autoantibodies; in the latter group, this genotype was

TT genotype frequency (0% and 13.3%, p=0.44), C peptide, DKA at onset,

and had lower levels of GAD65 autoantibodies (p=0.04) compared with lean

expressed a single islet autoantibody (n=11) had a signi-

expression of GAD65 or insulin auto-

mean (SD) 21.8±10.2

HbA1c (%) (mmol/mol): mean (SD) 12.1±2.2 (109±24)

Beta-hydroxybutyrate (mmol/L): mean (SD)

Expression of islet autoantibodies: n (%)

Number of islet autoantibodies expressed: n (%)

1 15 (21.1)

2 25 (35.2)

3 25 (35.2)

4 6 (8.5)

HLA DR3-DQ2/DR4-DQ8: n (%) 16 (22.5)

BMI, body mass index; DKA, diabetic ketoacidosis; GAD65, glutamic acid decarboxylase 65; HbA1c, glycated hemoglobin; HLA, human leukocyte antigen; ICA512/IA2, islet cell autoantigen 512; ZnT8, zinc transporter 8.

RESULTS

Mean age of the 71 children at diagnosis of autoimmune type 1 diabetes was 10.7±3.8 years. A single autoantibody was expressed by 15 (21.1%) of the children. Other demographic and clinical characteristics are depicted in table 1.

The distribution of TCF7L2 rs7903146 genotypes in children with a single islet autoantibody (n=15) was signifi-

Table 1 Characteristics of children at diagnosis of autoimmune type 1 diabetes (N=71)

Age (years): mean (SD) 10.7±3.8

Male gender: n (%) 43 (60.6)

Race/ethnicity: n (%) 0.05 were considered statistically signi

Type 1 diabetes was 10.7±3.8 years. A single autoantibody was expressed by 15 (21.1%) of the children. Other demographic and clinical characteristics are depicted in table 1.

The distribution of TCF7L2 rs7903146 genotypes in children with a single islet autoantibody (n=15) was signifi-

among children with a single autoantibody. Children who expressed a single islet autoantibody had lower frequency of DKA, lower serum concentrations of β-hydroxybutyrate at diagnosis, and were more likely to have elevated fasting C peptide levels (defined as values above the median of the logarithmic distribution of C peptide; figure 1).

Children who were lean (BMI<85th centile) and expressed a single islet autoantibody (n=11) had a significantly higher frequency of the TCF7L2 TT genotype (45.5%) compared with lean children with ≥2 positive autoantibodies; in the latter group, this genotype was completely absent (n=25, 0%, p<0.0001). Lean children with a single autoantibody were also more likely to have an elevated C peptide (72.7% vs 32.0%, p=0.023) and less likely to express ICA512/IA2 (p<0.0001), ZnT8 (p=0.01), and GAD65 autoantibodies (p=0.04) compared with lean children who expressed ≥2 autoantibodies. They tended to present less often with DKA (10% vs 39.1%, p=0.094) and had lower levels of β-hydroxybutyrate (p<0.03).

Among obese or overweight children (n=34), those with a single and ≥2 islet autoantibodies were not statistically different for TT genotype frequency (0% and 13.3%, respectively, p=0.44), C peptide, DKA at onset, β-hydroxybutyrate, or expression of GAD65 or insulin autoantibodies. Expression of ICA512/IA2 autoantibodies was lower (p<0.001), and that of ZnT8 autoantibodies tended to be lower (p=0.07) in single compared with ≥2 autoantibody-positive obese type 1 diabetes children.

Table 1 Characteristics of children at diagnosis of autoimmune type 1 diabetes (N=71)

<table>
<thead>
<tr>
<th>Age (years): mean (SD)</th>
<th>10.7±3.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender: n (%)</td>
<td>43 (60.6)</td>
</tr>
<tr>
<td>Race/ethnicity: n (%)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Non-Hispanic white 39 (54.9)

Hispanic 23 (32.4)

African-American 6 (8.5)

Other 3 (4.2)

BMI percentile: mean (SD) 76.2±22.3

Obesity or overweight: n (%) 34 (48.6)

Glucose (mmol/L): mean (SD) 21.8±10.2

HbA1c (%) (mmol/mol): mean (SD) 12.1±2.2 (109±24)

DKA: n (%) 26 (38.8)

Beta-hydroxybutyrate (mmol/L): mean (SD) 5.3±4.3

Fasting C peptide (mmol/L): median (25th–75th centiles) 0.143 (0.083–0.243)

Expression of islet autoantibodies: n (%)

Insulin 27 (38)

GAD65 41 (57.8)

ICA512/IA2 52 (73.2)

ZnT8 44 (62)

Number of islet autoantibodies expressed: n (%)

1 15 (21.1)

2 25 (35.2)

3 25 (35.2)

4 6 (8.5)

HLA DR3-DQ2/DR4-DQ8: n (%) 16 (22.5)

BMI, body mass index; DKA, diabetic ketoacidosis; GAD65, glutamic acid decarboxylase 65; HbA1c, glycated hemoglobin; HLA, human leukocyte antigen; ICA512/IA2, islet cell autoantigen 512; ZnT8, zinc transporter 8.
DISCUSSION

In the present study, we observed that the TCF7L2 locus is associated with single islet autoantibody expression in children diagnosed with autoimmune type 1 diabetes. Children with single autoantibody positivity were less likely to express ICA512/IA2 and ZnT8 autoantibodies, and had lower incidence of DKA and higher fasting C peptide levels than children with ≥2 positive autoantibodies. When the cohort was divided into lean and obese/overweight subgroups, most of these differences were present in the lean children but not among overweight or obese children. Almost half of the lean children with a single islet autoantibody were rs7903146 TT homozygotes, compared to none of the lean children with ≥2 positive autoantibodies.

Children with single islet autoantibody positivity had additional evidence of milder islet autoimmunity. They were less likely to present with DKA and had lower plasma concentration of β-hydroxybutyrate at diagnosis than children with ≥2 islet autoantibodies. Since DKA is a marker of near-complete insulin deficiency, these findings are consistent with less aggressive β-cell destruction. Indeed, compared with children with ≥2 positive autoantibodies, those with a single autoantibody had greater β-cell function, as measured by fasting serum C peptide. Single-autoantibody children were also less likely to express ICA512/IA2 and ZnT8 autoantibodies. Seroconversion of ICA512/IA2 and ZnT8 autoantibodies has been found to immediately precede progression to type 1 diabetes in subjects at risk,28 29 reflecting their association with aggressiveness of the autoimmune attack on β-cells.

Our finding that children with type 1 diabetes and mild islet autoimmunity were more likely to be carriers of the TCF7L2 rs7903146 TT genotype suggests that a TCF7L2-dependent mechanism may be contributing to their diabetes. The TCF7L2 protein is expressed in the β-cell and is involved in proliferation and apoptosis, insulin processing, and regulated expression of receptors.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Single positive autoantibody</th>
<th>≥2 Positive autoantibodies</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years): mean (SD)</td>
<td>11.7±4.6</td>
<td>10.4±3.6</td>
<td>0.25</td>
</tr>
<tr>
<td>Male gender: n (%)</td>
<td>9 (60)</td>
<td>34 (60.7)</td>
<td>0.96</td>
</tr>
<tr>
<td>Race/ethnicity: n (%)</td>
<td></td>
<td></td>
<td>0.89</td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>9 (60)</td>
<td>30 (53.6)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>4 (26.7)</td>
<td>19 (33.9)</td>
<td></td>
</tr>
<tr>
<td>African-American</td>
<td>1 (6.7)</td>
<td>5 (8.9)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1 (6.7)</td>
<td>2 (3.6)</td>
<td></td>
</tr>
<tr>
<td>BMI centile: mean (SD)</td>
<td>64.1±25.3</td>
<td>79.7±20.4</td>
<td>0.01</td>
</tr>
<tr>
<td>Obesity or overweight: n (%)</td>
<td>4 (26.7)</td>
<td>30 (54.6)</td>
<td>0.06</td>
</tr>
<tr>
<td>Glucose (mmol/L): mean (SD)</td>
<td>19.2±5.5</td>
<td>22.5±11.1</td>
<td>0.26</td>
</tr>
<tr>
<td>HbA1c (%): mean (mmol/mol)</td>
<td>12.6±2 (114±21.9)</td>
<td>11.9±2.2 (107±24)</td>
<td>0.29</td>
</tr>
<tr>
<td>DKA: n (%)</td>
<td>2 (14.3)</td>
<td>24 (45.3)</td>
<td>0.034</td>
</tr>
<tr>
<td>β-hydroxybutyrate (mmol/L):</td>
<td>2.6±2.3</td>
<td>6±4.4</td>
<td>0.006</td>
</tr>
<tr>
<td>Elevated* fasting C peptide: n%</td>
<td>11 (73.3)</td>
<td>24 (42.9)</td>
<td>0.036</td>
</tr>
<tr>
<td>Fasting C peptide (ng/mL):</td>
<td>1.19 (0.76)</td>
<td>0.67 (1.5)</td>
<td>0.96</td>
</tr>
<tr>
<td>Positive islet autoantibodies: n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>4 (26.7)</td>
<td>23 (41.1)</td>
<td>0.31</td>
</tr>
<tr>
<td>GAD65</td>
<td>6 (40)</td>
<td>35 (62.5)</td>
<td>0.12</td>
</tr>
<tr>
<td>ICA512/IA2</td>
<td>2 (13.3)</td>
<td>50 (89.3)</td>
<td>0.0001</td>
</tr>
<tr>
<td>ZnT8</td>
<td>3 (20)</td>
<td>41 (73.2)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Titers of islet autoantibodies: mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>0.46 (0.487)</td>
<td>1.617 (5.295)</td>
<td>0.4</td>
</tr>
<tr>
<td>GAD65</td>
<td>0.152 (0.256)</td>
<td>0.37 (0.603)</td>
<td>0.18</td>
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<tr>
<td>ICA512/IA2</td>
<td>0.115 (0.298)</td>
<td>0.617 (0.421)</td>
<td>0.0001</td>
</tr>
<tr>
<td>ZnT8Arg</td>
<td>19.6 (51.4)</td>
<td>161 (239.1)</td>
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<tr>
<td>ZnT8Trp</td>
<td>10.8 (24.6)</td>
<td>139.6 (263.6)</td>
<td>0.064</td>
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<tr>
<td>HLA DR3-DQ2/DR4-DQ8: n (%)</td>
<td>3 (20)</td>
<td>13 (23.2)</td>
<td>0.79</td>
</tr>
<tr>
<td>TCF7L2 SNP rs7903146 genotype: n (%)</td>
<td></td>
<td></td>
<td>0.024</td>
</tr>
<tr>
<td>CC</td>
<td>6 (40)</td>
<td>28 (50)</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>4 (26.7)</td>
<td>24 (42.9)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>5 (33.3)</td>
<td>4 (7.1)</td>
<td></td>
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</tbody>
</table>
*Above the median of the logarithmic distribution.

BMI, body mass index; DKA, diabetic ketoacidosis; GAD65, glutamic acid decarboxylase 65; HbA1c, hemoglobin A1c; HLA, human leukocyte antigen; ICA512/IA2, islet cell autoantigen 512; IQR, interquartile range; SNP, single nucleotide polymorphism; TCF7L2, transcription factor 7-like 2; ZnT8, zinc transporter 8.
children with autoimmune type 1 diabetes and, in particular, in relation to the severity of islet autoimmunity. Previous pediatric studies have reported that the type 2 diabetes-associated TCF7L2 gene polymorphism is more frequent in diabetes without evidence of islet autoimmunity, for example, in autoantibody-negative than autoantibody-positive type 1 diabetes children and in GAD65 autoantibody-negative than GAD65 autoantibody-positive patients aged 15–34. In contrast, we studied the contribution of TCF7L2 in well-defined subsets of children with autoimmune diabetes. One limitation of this study is the relatively small sample size, which may affect the generalizability of the study. Confirmation of our findings in an independent study sample is warranted. Another limitation is the use of expression of a single islet autoantibody to define mild islet autoimmunity. Although in our study the expression of a single islet autoantibody correlated with clinical and laboratory indicators of mild β-cell autoimmunity destruction, the identification of patients with mild islet autoimmunity may improve as the sensitivity and specificity of current autoantibody assays increase, and as better markers of the aggressiveness of the immune attack on β-cells are validated.

In summary, our findings that the type 2 diabetes-associated TCF7L2 variant is carried in the homozygote form by a third of children with type 1 diabetes and single islet autoantibody positivity (and almost half of the lean children with type 1 diabetes and a single islet autoantibody) support the hypothesis that nonautoimmune pathways may significantly contribute to diabetogenesis in well-defined subsets of pediatric autoimmune type 1 diabetes.

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Contributors MJR designed and conducted the study, analyzed and interpreted the data, and wrote the first draft of the manuscript. JMR conducted genetic testing and reviewed the manuscript. LMR, MW, and AB contributed to study design, data interpretation, and review of the manuscript. DI processed the serum and DNA samples, conducted C peptide testing, and reviewed the manuscript. FVS provided the ZnT8 construct and reviewed the

Figure 1 Percentages of children with autoimmune type 1 diabetes who carried the TCF7L2 rs7903146 TT genotype, expressed ICA512/IA2 or ZnT8 autoantibodies, presented with DKA and had elevated fasting C peptide levels 7 weeks after onset: comparison with children with a single versus ≥2 positive islet autoantibodies. Black columns=children expressing a single islet autoantibody. White columns= children expressing ≥2 positive islet autoantibodies. Aab, autoantibody; DKA, diabetic ketoacidosis; ICA512, islet cell autoantigen 512; TCF7L2, transcription factor 7-like 2; ZnT8, zinc transporter 8.
Genetics/genomes/proteomics/metabolomics

manuscript. CSH oversaw autoantibody testing and contributed to data interpretation and review of the manuscript. MLM oversaw genetic testing and contributed to data interpretation and review of the manuscript. SFAG contributed to data interpretation and review of the manuscript. MJR is the guarantor.

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Competing interests None.

Patient consent Obtained.

Ethics approval Baylor College of Medicine IRB.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

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REFERENCES