Aspects of ZnT8 autoimmunity in childhood type 1 diabetes

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Original Article

Triple specificity of ZnT8 autoantibodies in relation to HLA and other islet autoantibodies in childhood and adolescent type 1 diabetes


Objective: To establish the diagnostic sensitivity of and the relationships between autoantibodies to all three Zinc transporter 8 (Zinc transporter 8 autoantibody to either one, two, or all three amino acid variants at position 325, ZnT8A) variants to human leukocyte antigen (HLA)-DQ and to autoantibodies to glutamic acid decarboxylase (GADA), insulinoma-associated protein 2 (IA-2A), and insulin (IAA).

Methods: We analyzed 3165 patients with type 1 diabetes (T1D) in the Better Diabetes Diagnosis study for HLA-DQ genotypes and all six autoantibodies (ZnT8RA, arginine 325 Zinc transporter 8 autoantibody; ZnT8WA, tryptophan 325 Zinc transporter 8 autoantibody; ZnT8QA, glutamine 325 Zinc transporter 8 autoantibody; GADA, IA-2A, and IAA).

Results: ZnT8A was found in 65% of the patients and as many as 108 of 3165 (3.4%) had 1–3 ZnT8A alone. None had ZnT8QA alone. Together with GADA (56%), IA-2A (73%), and IAA (33%), 93% of the T1D patients were autoantibody positive. All three ZnT8A were less frequent in children below 2 yr of age (p < 0.0001). All three ZnT8A were associated with DQA1-B1*X-0604 (DQ6.4) and DQA1-B1*03-0302 (DQ8). ZnT8WA and ZnT8QA were negatively associated with DQA1-B1*05-02 (DQ2).

Conclusions: Analysis of ZnT8A increased the diagnostic sensitivity of islet autoantibodies for T1D as only 7% remained islet autoantibody negative. The association between DQ6.4 and all three ZnT8A may be related to ZnT8 antigen presentation by the DQ6.4 heterodimer.

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†Members of the BDD Study group are listed in the Appendix.

Key words: diabetes mellitus – GAD65 autoantibodies – HLA genotype – IA-2 autoantibodies – insulin autoantibodies – type 1 diabetes – Zinc transporter – ZnT8

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At the time of clinical diagnosis of type 1 diabetes (T1D), most patients (90–97%) (1–3) display autoantibody markers for T1D. Autoantibodies to glutamic acid decarboxylase 65 (GADA), insulin (IAA), and insulinoma-associated protein 2 (IA-2A) together have been reported to have a diagnostic sensitivity of 50–80% (3, 4).

The Zinc transporter 8 (ZnT8) is a β-cell granule protein recently shown to be an autoantigen in T1D (2, 4). Zinc is highly concentrated in the secretory vesicles of islet β-cells, where it is required for crystallization of insulin. ZnT8 is expressed in β cells (5), peripheral blood lymphocytes (6), subcutaneous fat tissue (7), and pancreatic α-cells (8). ZnT8 is also expressed in cubical cells lining the thyroid follicle and in adrenal cortex cells (9). Autoantibodies against the ZnT8 differentiate between three different variants at position 325 in the protein (ZnT8RA, arginine 325 Zinc transporter 8 autoantibody; ZnT8WA, tryptophan 325 Zinc transporter 8 autoantibody; and ZnT8QA, glutamine 325 Zinc transporter 8 autoantibody). Autoantibodies to any of these three variants represent additional immunological markers of T1D risk (2, 4). The human leukocyte antigen (HLA)-DQA1*0501-DQB1*0201 (DQ2) and HLA-DQA1*0301-DQB1*0302 (DQ8) haplotypes confer susceptibility in T1D (10, 11). The most common HLA genotype in T1D is HLA-DQA1*0501-DQB1*0201/DQA2*0301-DQB2*0302 (DQ2/8) (10, 12). Regardless of HLA-DQ (13), the number of islet autoantibodies appears to be associated with an increased risk for clinical onset of T1D and is the best known predictor of T1D (11, 14–16). The aims of this study were as follows: (i) to establish the diagnostic sensitivity of currently available autoantibodies (GADA, IA-2A, and IAA along with all three ZnT8RA, ZnT8WA, and ZnT8QA) against known islet autoantigens; (ii) to determine whether the number of islet autoantibodies was associated with age at diagnosis; and (iii) to analyze the association between HLA-DQ and islet autoantibodies.

Subjects and methods

Subjects
Sweden has 9.4 million inhabitants who are served by 42 clinical centers for pediatric diabetes where all children with diabetes are diagnosed and treated. Better Diabetes Diagnosis (BDD) is a nationwide prospective study for newly diagnosed T1D patients who are <18 yr old. Blood samples at diagnosis, before insulin, or within 3 d after diagnosis were obtained between May 2005 and August 2010 for HLA genotyping and analyzed for GADA, IA-2A, and IAA and all three Zinc transporter 8 autoantibody to either one, two, or all three amino acid variants at position 325 (ZnT8A). The classification was reevaluated each year through the SWEDIAB KIDS register, a nationwide register for childhood and adolescent diabetes, making it possible for the clinicians to reclassify the patients for 1–5 yr after diagnosis (17). A total of 3719 patients were analyzed and 3165 (56% boys) classified as T1D according to ADA recommendations (18), whereas 58 were classified as type 2 diabetes, 33 as MODY, 9 as secondary diabetes mellitus, and 326 as unclassifiable type of diabetes mellitus at the time of clinical diagnosis (Fig. 1). In addition, there were 128 patients who had to be excluded because of missing data or serum. The group of 326 unclassifiable type of diabetes likely includes patients with T1D (19) but were excluded from this study as the final classification was missing. The median age of the children at T1D diagnosis was 10.1 yr (range 0–17.9 yr) (10.6 for boys and 9.7 for girls). Serum samples for the islet autoantibody assays were stored at −20°C until analyzed.

The study was approved by the Regional Ethical Review Board in Stockholm, Sweden.

Autoantibodies to the Zinc transporter variants
The radiobinding assay for all three variants, ZnT8R, ZnT8W, and ZnT8Q, was performed separately as described (20). The results were expressed in arbitrary units derived from in-house positive and negative standard samples. Intra-assay coefficient of variation (CV) for the ZnT8RA was 6%, ZnT8WA 5%, and ZnT8QA 4%. Inter-assay CV for ZnT8RA was 7%, ZnT8WA 8%, and ZnT8QA 10%. Our laboratory has been validated in Diabetes Autoantibody Standardization Program (DASP) 2011 with 50% study sensitivity and 100% study specificity for ZnT8RA, 46% study sensitivity and 100% study specificity for ZnT8WA, and 38% study sensitivity and 100% study specificity for ZnT8QA (21). Cutoff values for ZnT8RA were ≥75 U/mL, ZnT8WA ≥75 U/mL, and ZnT8QA ≥100 U/mL.

GAD65 and IA-2 autoantibodies
Recombinant GAD65 and IA-2 were labeled with 35S-methionine (GE Healthcare Life Sciences, Amersham, UK) by in vitro coupled transcription and translation in TNT SP6 coupled reticulocyte lysate system (Promega, Southampton, UK) as described (22). Full-length cDNA coding for human GAD65 in the pTNT vector (Promega) (pThGAD65) or the intracellular domain (amino acids 603–980) of IA-2 in the pSP64 Poly(A) vector (Promega) (IA-2ic) was used (23). GADA and IA-2A were analyzed in a radioligand binding assay (22) in samples eluted from dried blood
ZnT8 autoantibodies and HLA in type 1 diabetes

Total
n=3719

Secondary diabetes mellitus
n=9 (0.2%)

Missing data
n=128 (3%)

Study group
n=3165 (85%)

5 Abs
n=489 (15%)

4 Abs
n=562 (18%)

3 Abs
n=582 (18%)

2 Abs
n=648 (20%)

1 Ab
n=482 (15%)

0 Ab
n=216 (7%)

Type 2 diabetes mellitus
n=58 (2%)

MODY
n=33 (1%)

Unclassifiable type of diabetes at diagnosis
n=326 (9%)

6 Ab-pos
52 Ab-neg

9 Ab neg

Insulin autoantibodies

IAA were determined in a non-competitive radioligand binding assay using $^{125}$I-insulin essentially as described (26). Duplicates of patient sera (7 μL) were incubated for 48 h at +4°C with 38 000 cpm of $^{125}$I-labeled human recombinant insulin (NEX420050UC, Perkin Elmer) in Tris buffer (pH 8.0) with 1% Tween 20, giving a total volume of 44 μL. The samples were transferred to filtration plates (Millipore) and free $^{125}$I-labeled insulin separated from antibody bound with Protein A-Sepharose (Zymed Laboratories Inc.). After washing with TBST, the plates were allowed to dry. Supermix scintillation cocktail (Perkin Elmer) was added and the radioactivity of antibody-bound $^{125}$I-labeled insulin counted in a Wallac Microbeta Trilux (Perkin Elmer) beta counter. All samples with IAA higher than 0.8 relative unit (RU) were further analyzed in a competitive radioligand binding assay. The samples (7 μL) were added to four wells in a 96-well plate. To all four wells, 36 μL of 38 000 cpm of $^{125}$I-labeled insulin was added, with an addition of 0.072 IU non-radioactive insulin in the last two wells. The

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Fig. 1. Flow chart of the Better Diabetes Diagnosis (BDD) study.

spots (DBSs). Discs at the size of 6 mm in diameter were punched (Wallac DBS puncher, PerkinElmer Life and Analytical Sciences, Brussels, Belgium) from DBS on filter paper (grade 2992 filters; Schleicher and Schuell, Dassel, Germany). The DBS discs were incubated overnight at +4°C in 80 μL Tris-buffered saline with Tween 20 (TBST) with shaking to elute antibodies. In the autoantibody assays, 30 μL DBS eluate was incubated with 24 000 cpm of $^{35}$S-labeled GAD65 or IA-2 in TBST in a final reaction volume of 60 μL. The samples were transferred to filtration plates (Millipore, Solna, Sweden) and free $^{35}$S-labeled GAD65 or IA-2 separated from antibody bound with Protein A-Sepharose (Zymed Laboratories Inc., San Francisco, CA, USA). After washing with TBST, the plates were allowed to dry. Supermix scintillation cocktail (Perkin Elmer, Boston, MA, USA) was added and the radioactivity of antibody-bound $^{35}$S-labeled GAD65 or IA-2 counted in a Wallac Microbeta Trilux (Perkin Elmer) beta counter. GADA and IA-2A levels were expressed as units per mL (U/mL) derived from the WHO standard 97/550 (24). Samples were considered positive if GADA levels were above 50 U/mL and IA-2A levels above 10 U/mL. The intra-assay CV for duplicates in the GAD65A assay was 7% and in the IA-2A 11%. In the DASP 2009 workshop, our laboratory had a 68% workshop sensitivity and 99% specificity for GADA and a 60% workshop sensitivity and 99% specificity for IA-2A (25).
final titer of IAA is calculated by subtracting the radioactivity in the wells with non-radioactive insulin from those with only $^{125}$I-labeled insulin. The results were expressed in arbitrary units derived from in-house positive and negative standard samples. Samples were considered positive if IAA levels were above 1 RU. Intra-assay CV was 6% and inter-assay CV was 13%. Our laboratory has been validated in DASP 2010 with 26% study sensitivity and 100% study specificity (25).

**HLA genotyping**

HLA-DQB1 and DQA1 genotypes were typed by sequence-specific oligonucleotide probes on DBS used directly for PCR amplification of DQA1 and DQB1 alleles as described (27) using a DELFIA Hybridization assay (Perkin Elmer). The first set of probes defines the presence of HLA-DQB1*02, 0302, 0301, 0602, 0603, and 0604. The second set of probes defines the presence of additional DQB1 alleles. HLA-DQA1 probes defines the presence of HLA-DQA1*0201, 03, and 05 alleles. The HLA frequencies of the patients were compared to 2000 newborn children in the Diabetes Prediction in Skåne (DiPiS) study (28, 29).

**Statistical methods**

Statistical analyses were performed using spss statistical software (version 18.0; SPSS, Chicago, IL, USA). Differences in proportions between groups were tested using the chi-squared test or Fisher’s exact test when appropriate. Bonferroni correction was used to correct for the number of comparisons. Odds ratios (ORs) with 95% confidence interval were calculated from simple logistic regression models to evaluate the degree of association between the categorical variables; p-values less than 0.05 were considered significant.

**Results**

ZnT8A are common at the time of clinical diagnosis

Among the 3165 patients studied at the time of T1D diagnosis and taking all three ZnT8A as one group, 2064 (65%) were positive. ZnT8RA was found among 54%, ZnT8WA among 47%, and ZnT8QA among 32% (data not shown). The Venn diagram analysis of ZnT8A without the other islet autoantibodies demonstrates that the three different ZnT8A may occur both alone and in different combinations (Fig. 2). A total of 39% were single positive representing 25% for ZnT8RA, 15% for ZnT8WA, and only 0.1% for ZnT8QA. All three ZnT8A were found in as many as 44% of the ZnT8A-positive patients compared to 17% with only two ZnT8A (Fig. 2).

ZnT8A is increasing the diagnostic sensitivity of islet autoantibodies for T1D

The diagnostic sensitivity for T1D of GADA, IA-2A, and IAA but without ZnT8A was 90% among the 3165 patients. When ZnT8A was included, the most common islet autoantibody was IA-2A (73%) followed by GADA (56%), ZnT8RA (54%), ZnT8WA (47%), IAA (33%), and ZnT8QA (32%) (data not shown). Among the 482 patients with only one islet autoantibody, IA-2A was found in 205 (43%), GADA in 181 (38%), ZnT8RA in 41 (9%), IAA in 38 (8%), and ZnT8WA in 17 (4%). One (n = 58), two (n = 21), or all three (n = 29) ZnT8A without any of the other islet autoantibodies were found in 108 of 3165 (3.4%) patients. In addition to the patients with all three (n = 29), the distribution of ZnT8A was as follows: ZnT8RA (n = 41), ZnT8WA (n = 17), both ZnT8RA and ZnT8WA (n = 9), ZnT8RA and ZnT8QA (n = 5), as well as ZnT8WA and ZnT8QA (n = 7). No patient was diagnosed with T1D with only ZnT8QA, but ZnT8QA was found as the only ZnT8A in two patients with only IA-2A without the ZnT8A analysis and in one patient with only IAA.

Number and combinations of islet autoantibodies

The number of islet autoantibodies was next analyzed. Prior to the analysis of ZnT8A, the distribution of the three islet autoantibodies (GADA, IA-2A, and IAA) showed that double autoantibody positivity was most common (39%). However, adding the ZnT8A to the analysis decreased the double autoantibody positivity to 17% along with a decrease in single autoantibody positivity from 29 to 18%. It is noted that 216 of...
Table 1. Relationship between islet autoantibodies and the risk (OR) for another islet autoantibody

<table>
<thead>
<tr>
<th></th>
<th>ZnT8WA</th>
<th>ZnT8QA</th>
<th>GADA</th>
<th>IA-2A</th>
<th>IAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnT8RA OR</td>
<td>6.4</td>
<td>43.1</td>
<td>1.4</td>
<td>3.2</td>
<td>1.2</td>
</tr>
<tr>
<td>CI</td>
<td>5.5–7.5</td>
<td>31.2–59.4</td>
<td>1.2–1.6</td>
<td>2.7–3.8</td>
<td>1.1–1.4</td>
</tr>
<tr>
<td>p-value</td>
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<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.005</td>
</tr>
<tr>
<td>ZnT8WA OR</td>
<td>40.0</td>
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</tr>
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<td>CI</td>
<td>30.7–52.0</td>
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<td>0.0004</td>
<td>&lt;0.0001</td>
<td>1.1–1.5</td>
</tr>
<tr>
<td>p-value</td>
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<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>ZnT8QA OR</td>
<td>1.3</td>
<td>0.95–1.3</td>
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<td>1.5</td>
<td></td>
</tr>
<tr>
<td>CI</td>
<td>1.1–1.5</td>
<td>2.5–3.7</td>
<td>0.2</td>
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<td>p-value</td>
<td>0.0006</td>
<td>0.0006</td>
<td>1.6–2.3</td>
<td>&lt;0.0001</td>
<td></td>
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<tr>
<td>GADA OR</td>
<td>1.1</td>
<td>1.1–1.3</td>
<td>1.1</td>
<td>1.5</td>
<td></td>
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<td>CI</td>
<td>0.9–1.3</td>
<td>2.5–3.7</td>
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<td>&lt;0.0001</td>
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<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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</tr>
<tr>
<td>IA-2A OR</td>
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<td>1.6–2.3</td>
<td>1.1</td>
<td>1.5</td>
<td></td>
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<tr>
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<td>p-value</td>
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<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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</tbody>
</table>

CI, confidence interval; GADA, autoantibodies to glutamic acid decarboxylase; IAA, insulin autoantibodies; IA-2A, insulinoma-associated protein 2 autoantibodies; OR, odds ratio; ZnT8QA, glutamine 325 Zinc transporter 8 autoantibody; ZnT8RA, arginine 325 Zinc transporter 8 autoantibody; ZnT8WA, tryptophan 325 Zinc transporter 8 autoantibody.

324 (67%) of the islet autoantibody-negative patients remained negative also after adding the ZnT8A. The presence of two or three autoantibodies of GADA, IA-2A, or IAA increased the likelihood to be diagnosed for T1D with ZnT8A.

We next analyzed the combinations of all six islet autoantibodies among the 2949 islet autoantibody-positive T1D patients. ZnT8RA and ZnT8QA were strongly associated (OR: 43) followed by ZnT8WA and ZnT8QA (OR: 40) (Table 1). All three ZnT8A were more strongly associated with IA-2A than GADA and IAA (Table 1).

The diagnostic sensitivity of islet autoantibodies and age at diagnosis

Analysis of the different islet autoantibodies at each year of age (Fig. 3) showed that ZnT8RA, ZnT8WA, and ZnT8QA increased from low frequencies in the 0–1.9 yr olds (p < 0.0001 for all three after correction for multiple comparisons) and 0–4.9 yr olds (p < 0.001 for all three after correction for multiple comparisons) compared to the children older than 2 or 5 yr of age, respectively. After 5 yr of age, the frequency of ZnT8RA, ZnT8WA, and ZnT8QA, respectively, reached a steady-state level (Fig. 3). As expected, IAA were more frequent among children below 5 yr of age (339/549; 62%) compared to the patients above 15 yr of age (81/427; 19%; p < 0.0001). GADA were also less frequent in the group of less than 10 yr of age (50%) compared to patients older than 10 yr of age (62%; p < 0.0001). There was little variation in the frequency of IA-2A (mean 73%) in relation to age at diagnosis (Fig. 3).

The frequency of 2–5 islet autoantibodies was independent of the age at diagnosis. It was noted that islet autoantibody-negative patients were more common above (10%) compared to below (6%; p = 0.004) 15 yr of age.

HLA-DQ and ZnT8 autoantibodies

The association between HLA-DQ genotypes and T1D is summarized in Supporting Information Table S1 also showing the number of islet autoantibodies in each genotype. Further analyses revealed that ZnT8RA was associated with both HLA-DQA1*X-DQB1*0604 (DQ6.4) and DQ8 but negatively associated with DQ2/8 but not associated with DQ2 (Table 2). ZnT8WA and ZnT8QA were associated with DQ8 and DQ6.4 but negatively associated with DQ2 and DQ2/8. We confirmed that GADA was associated with DQ2 but also with DQ2/8 and negatively associated with DQ8. IA-2A was associated with DQ8 and negatively...
Table 2. Associations between HLA-DQ genotypes and all islet autoantibodies at diagnosis of type 1 diabetes in 3165 children

<table>
<thead>
<tr>
<th></th>
<th>ZnT8RA</th>
<th>ZnT8WA</th>
<th>ZnT8QA</th>
<th>GADA</th>
<th>IA-2A</th>
<th>IAA</th>
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<td><strong>DQ 2/2 and 2/X</strong></td>
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<td></td>
<td></td>
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<tr>
<td>(X is not 8)</td>
<td>OR</td>
<td>CI</td>
<td>p-value</td>
<td>OR</td>
<td>CI</td>
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<tr>
<td></td>
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<td>0.8</td>
<td>0.0005</td>
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Cl, confidence interval; GADA, autoantibodies to glutamic acid decarboxylase; HLA, human leukocyte antigen; IAA, insulin autoantibodies; IA-2A, insulinoma-associated protein 2 autoantibodies; OR, odds ratio; ZnT8QA, glutamine 325 Zinc transporter 8 autoantibody; ZnT8RA, arginine 325 Zinc transporter 8 autoantibody; ZnT8WA, tryptophan 325 Zinc transporter 8 autoantibody.

Dark shade represents a positive association; light shade represents a negative association.

Our study demonstrates that adding ZnT8A to the prior battery of GADA, IA-2A, and IAA reduced the frequency of islet autoantibody-negative patients from 10 to 7% – a reduction by 33%. Hence, the diagnostic sensitivity increased from 90 to 93% in a nationwide patient population, clinically classified at diagnosis and with confirmation of classification after 1–5 yr. The BDD study ascertained almost all children in Sweden who were diagnosed with diabetes in 2005–2010. In addition, all BDD patients were cross-referenced to the Swedish quality register SWEDIABKIDS (17) to secure complete ascertainment and quality of classification. Representing a high-incidence country such as Sweden as many as 3719 children were ascertained, which made it possible to analyze age at diagnosis data as year of age. We could confirm that the contribution of ZnT8A to T1D below the age of 2 was limited (4). Multiple autoantibodies were common at all ages and as common in children younger than 2 yr of age. The well-known decline in IAA frequency with increasing age at diagnosis was pronounced in this study (Fig. 3). Islet autoantibody-negative patients were more common below the age of 2 as well as above the age of 15. However, in 1–2 yr of age, the frequencies could vary because of smaller number of patients. Children with six islet autoantibodies showed similar frequencies as the islet autoantibody-negative patients (Fig. 1).

Our analyses of associations between islet autoantibodies demonstrate a strong association between all three variants of ZnT8A.
ZnT8 autoantibodies and HLA in type 1 diabetes

Fig. 4. Algorithm for classification of diabetes in the 0–18 yr olds.

possibly, DQ2 (Table 2). Our data therefore suggest that the association between HLA-DQ 6.4/8 and 6.4/2 and T1D is secondary to a primary association between DQ6.4 and all ZnT8A, regardless of the polymorphic amino acid at position 325.

The observation that DQ 8/8 and 8/X (X is not DQ2) was positively associated with ZnT8RA, ZnT8WA, and ZnT8QA supports the possible importance of the DQ8 heterodimer for ZnT8 autoantigen presentation (31). In contrast, a negative association between DQ2/2 and 2/X (X is not DQ8) was only observed for ZnT8WA and ZnT8QA but not for ZnT8RA. It was possible to detect these novel observations in this study of 3165 T1D patients from all over Sweden in contrast to our former investigation of the Skåne county T1D children (13). We suggest that the negative association, i.e., inhibition of ZnT8 autoantibody formation, dominates over the DQ8-mediated antigen presentation as the heterozygous DQ2/8 patients were negatively associated with all three ZnT8A variants. Furthermore, the negative association with DQ2/8 may also explain the low frequency of ZnT8A in the very young as the DQ2/8 genotype dominates in this age group. In children older than 2 yr of age, however, the presence of one, two, or all three ZnT8A, found among 65% of the patients, was comparable to the frequencies in recent studies (13, 15, 32).

ZnT8QA was not detected in any patient as the only islet autoantibody, and in our previous study, only one patient had ZnT8QA as the single islet autoantibody (13). We suggest that ZnT8QA is the least cost-efficient islet autoantibody to analyze to improve diagnostic sensitivity. As in our previous investigation of T1D children from the Skåne county, we also applied Kappa statistics (33) to the data in this study (data not shown). This analysis revealed that ZnT8QA is common together with the other two ZnT8A. We also found that ZnT8QA exists as the only ZnT8A in combination with IA-2A or IAA, which makes it useful also to analyze ZnT8QA for prediction. The Kappa statistics demonstrate that analyses of the different variants of ZnT8A cannot replace each other.

On the basis of our Swedish data from this population-based and countrywide study on 0- to 18-yr-old diabetes patients, we have implemented the following algorithm for classification of childhood diabetes (Fig. 4). The sample obtained at diagnosis is analyzed for GADA, IA-2A, and HLA. In Sweden, we consider HLA typing in step 1 important for risk evaluation of T1D as well as celiac disease. Samples negative for GADA and IA-2A are next analyzed for IAA and triple ZnT8A (20). We have found that about 18% will reach the second step in Swedish patients. In step 3, samples negative for both IAA and ZnT8A (about 11% of Swedish clinical onset samples) will first be retested for GADA, IA-2A, and ZnT8A in follow-up samples during the first year of diabetes. These analyses are done as 6 of 52 (12%) seroconverted during the first
year of diabetes (data not shown). The remaining group will be analyzed for monogenic diabetes (including MODY), T2D, and secondary diabetes (Fig. 4). It should be noted that secondary diabetes most likely has already been identified.

The only autoantibody not included in this algorithm is islet cell antibody (ICA). In the Skåne study, we found that 10 of 686 (1.5%) of the T1D patients had ICA but no other islet autoantibody (13). A potential weakness of the present classification approach is that some patients may be ICA positive but negative for all other autoantibodies. In addition, it cannot be excluded that some of these patients may have autoantibodies to less frequent autoantigens (34).

We conclude from this study that ZnT8A were common in newly diagnosed T1D independent of age and increased the diagnostic sensitivity of islet autoantibodies for T1D, as only 7% remained autoantibody negative. As many as 108 children (3.4%) had only ZnT8A at diagnosis. However, the contribution of ZnT8 autoimmunity to T1D below the age of 2 was limited. All variants of ZnT8A were positively associated with either DQ6.4 or DQ8 but dominantly negative with DQ2. Analysis of ZnT8A in relation to the immunogenetic risk is important to uncover the mechanisms by which ZnT8 autoimmunity contributes to T1D. The ZnT8A may also be important for prediction of childhood T1D and to randomize subjects in prevention and intervention clinical trials.

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Conflict of interest

None

Author contributions

CA analyzed and interpreted data and wrote the manuscript, FV-S contributed with development of the ZnT8A analysis and method description and edited the manuscript, AJD provided data, designed and updated the database for the BDD study, and edited the manuscript, BL, AC, GF, JL, CM, and US contributed to the study design and edited the manuscript, SAI, AL, and HE Larsson designed the study, interpreted data, and contributed to and edited the manuscript. All authors approved the final version to be published.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. HLA-DQ genotypes, the number of islet autoantibodies, and OR, 95th CI at diagnosis of T1D in 3165 children. The HLA frequencies were compared to 2000 newborn children in the Diabetes Prediction in Skåne (DiPiS) study (28, 29).

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References


Appendix

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