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Andersson, Cecilia K; Larsson, Karin; Vaziri Sani, Fariba; Lynch, Kristian; Carlsson, Annelie; Cedervall, E; Jönsson, B; Neiderud, J; Månsson, Majvi; Nilsson, Anna-Lena; Lernmark, Åke; Larsson, Helena; Ivarsson, Sten

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THE THREE ZNT8 AUTOANTIBODY VARIANTS TOGETHER IMPROVE THE DIAGNOSTIC SENSITIVITY OF CHILDHOOD AND ADOLESCENT TYPE 1 DIABETES

C. Andersson[1*], K. Larsson[2], F. Vaziri-Sani[1], K. Lynch[1], A. Carlsson[1], E. Cedervall[3], B. Jönsson[4], J. Neiderud[5], M. Månsson[1], A. Nilsson[1], Å. Lernmark[1], H. Elding Larsson[1] and S-A. Ivarsson[1]

[1] Department of Clinical Sciences, Skåne University Hospital SUS, Lund University, CRC, Malmö, Sweden

[2] Department of Paediatrics, Kristianstad Hospital, Kristianstad, Sweden

[3] Department of Paediatrics, Ängelholm Hospital, Ängelholm, Sweden

[4] Department of Paediatrics, Ystad Hospital, Ystad, Sweden

[5] Department of Paediatrics, Helsingborg Hospital, Helsingborg, Sweden

*Corresponding author:
Cecilia Andersson
Lund University/CRC,
Department of Clinical Sciences,
Entrance 72, Bldg 91, level 10,
Skåne University Hospital SUS, Malmö
SE-20502 Malmö
Sweden
Telephone +46 40 39 11 12
Fax +46 40 39 19 19
e-mail: Cecilia_K.Andersson@med.lu.se

Abstract

Aims We tested whether autoantibodies to all three ZnT8RWQ variants, GAD65, insulinoma-associated protein 2 (IA-2), insulin and autoantibodies to islet cell cytoplasm (ICA) in combination with human leukocyte antigen (HLA) would improve the diagnostic sensitivity of childhood type 1 diabetes by detecting children who otherwise would have been autoantibody negative.

Methods A total of 686 patients diagnosed 1996-2005 in Skåne were analyzed for all seven autoantibodies [arginine 325 zinc transporter 8 autoantibody (ZnT8RA), tryptophane 325 zinc transporter 8 autoantibody (ZnT8WA), glutamine 325 zinc transporter 8 autoantibody (ZnT8QA), autoantibodies to glutamic acid decarboxylase (GADA), autoantibodies to islet-antigen-2 (IA-2A), insulin autoantibodies (IAA) and ICA] in addition to HLA-DQ genotypes.

Results Zinc transporter 8 autoantibody to either one or all three amino acid variants at position 325 (ZnT8RWQA) was found in 65% (449/686) of the patients. The frequency was independent of age at diagnosis. The ZnT8RWQA reduced the frequency of autoantibody-negative patients from 7.5% to 5.4% - a reduction by 28%. Only 2/108 (2%) patients who are below 5 years of age had no autoantibody at diagnosis. Diagnosis without any islet autoantibody increased with increasing age at onset. DQA1-B1*X-0604 was associated with both ZnT8RA (p=0.002) and ZnT8WA (p=0.01) but not with ZnT8QA (p=0.07). Kappa agreement analysis showed moderate (>0.40) to fair (>0.20) agreement between pairs of autoantibodies for all combinations of GADA, IA-2A, ZnT8RWQA and ICA but only slight (<0.19) agreement for any combination with IAA.

Conclusions This study revealed that (1) the ZnT8RWQA was common, independent of age; (2) multiple autoantibodies were common among the young; (3) DQA1-B1*X-0604 increased the risk for ZnT8RA and ZnT8WA; (4) agreement between autoantibody pairs was common for all combinations except IAA. These results suggest that ZnT8RWQA is a necessary complement to the classification and prediction of childhood type 1 diabetes as well as to randomize subjects in the prevention and intervention of clinical trials.

Keywords

Diabetes mellitus
GAD65 autoantibodies
Human leukocyte antigen genotype
IA-2 autoantibodies
Islet cell cytoplasm
Insulin autoantibodies

Abbreviations

GADA, autoantibodies to glutamic acid decarboxylase;
ICA, autoantibodies to islet cell cytoplasm;
IAA, insulin autoantibodies;
IA-2A, insulinoma-associated protein 2 autoantibodies;
T1D, type 1 diabetes mellitus;
ZnT8RA, Arginin 325 Zinc transporter 8 autoantibody;
ZnT8WA, Tryptophan 325 Zinc transporter 8 autoantibody;
ZnT8QA, Glutamine 325 Zinc transporter 8 autoantibody;
ZnT8RWQA, Zinc transporter 8 autoantibody to either one or all three amino acid variants at position 325.

Introduction

Type 1 diabetes mellitus (T1D) is one of the most common chronic disorders in children, affecting more than 1% of the general population in Sweden. T1D is a multi-factorial autoimmune disease, with a strong genetic predisposition. The concordance rate of siblings of T1D patients is 6.4-6.7% [1, 2] and for identical twins less than 50% [3], indicating the importance of environmental factors for the development of the disease. The genetic susceptibility for T1D is primarily associated with the human leukocyte antigen (HLA) DQ locus on chromosome 6p21 [1, 4-6]. Recent Genome-wide association studies suggest that 30 or more genetic factors, mostly found to be expressed in immune system cells, may contribute additional risks [7].

The highest risk HLA genotype for development of T1D is HLA DQ A1*-B1*0501-0201/03-0302 or DQB1*0302/X, where X is neither 0602 nor 0603 [6, 8]. Both 0602 and 0603 along with 0301 were negatively associated with T1D [4, 6, 8, 9]. However, considering both the genotypes, the X-0501, X-0604 and X-04 haplotypes in combination with either high risk 03-0302 or 0501-0201 haplotypes represent risk genotypes for T1D [10]. This is important as age at onset have been inversely related to the proportion of HLA high-risk susceptibility genotypes [11] and that young children with T1D show the highest frequency of the 0501-0201/03-0302 genotype [12]. HLA alone does not predict T1D but rather indicates an increased predisposition for the development of islet autoimmunity that precedes T-cell mediated destruction of the pancreatic islet beta cells [6, 13] Autoantibodies to islet cell cytoplasm (ICA), insulin autoantibodies (IAA), autoantibodies to glutamic acid decarboxylase 65 (GADA), insulinoma-associated protein 2 autoantibodies (IA-2A) and zinc transporter 8 (ZnT8RA, arginin 325 zinc transporter 8 autoantibody; ZnT8WA, tryptophan 325 zinc transporter 8 autoantibody; ZnT8QA, glutamine 325 zinc transporter 8 autoantibody) precede clinical T1D by several years and thus represent immunological markers of T1D risk [14]. The risk of progression to T1D increases with the number of autoantibodies [15-18]. Although major research interests in T1D are focused on children at risk, the clinical reality is still that the vast majority of children are first seen at the time when symptoms of diabetes appear. As the frequency of children diagnosed with diabetes in conjunction with ketoacidosis tends

to decrease [19], it may sometimes be unclear if the patient has T1D at the time of presentation. Analyzing islet autoantibodies at diagnosis should prove helpful in this respect. However, as many as 10% of new T1D patients may be negative for any of ICA, IAA, GADA or IA-2A. It has been suggested that the addition of ZnT8RA, ZnT8WA and ZnT8QA may improve the diagnostic sensitivity particularly in adults [20-25]
The first aim of this study is to determine the diagnostic sensitivity of all three autoantibodies ZnT8RA, ZnT8WA and ZnT8QA and their relation to the other islet autoantibodies and HLA. Our second aim is to test if HLA DQ A1-B1 alleles, haplotypes or genotypes were associated with any of the ZnT8 autoantibodies.

Subjects And Methods

Subjects The province Skåne in the south part of Sweden has 1.200 000 inhabitants who are served by six clinical centers for pediatric diabetes where all children with T1D are diagnosed and treated. Serum samples at diagnosis were obtained between January 1996 and April 2005 from 686 consecutive patients who were classified with T1D according to the recommendation by ADA [26]. There were 373 (54%) boys and 313 (46%) girls in the study. The mean age of the children at T1D diagnosis was 9.8 years. Serum samples for the islet autoantibody assays were stored at –20˚C until analyzed.

The study was approved by the Human Research Ethics Committee of the Faculty of Medicine, Lund University, Lund, Sweden.

Autoantibodies to ICA were determined in a two-colour indirect immunofluorescence assay performed on sections of frozen human pancreas, as described previously [27] Our laboratory participated in the 13th immunology of diabetes workshop standardization and showed sensitivity and specificity of 100%. Levels of ICA are expressed in Juvenile Diabetes Foundation Units (JDF-U), using the world reference standard curve based on the international JDF reference sera sample.

Autoantibodies to GAD were analyzed using a commercially available kit for radioligand-binding assay for GADA autoantibodies according to the instructions given by the manufacturer (RSR Limited, Cardiff, UK). The RSR kit has been validated in the Diabetes Autoantibody Standardization Program (DASP) with 74% study sensitivity and 96% study specificity. The coefficient of variation (CV) was 8.9% at level 2.0 Units/mL and 14.2% at level 44.6 units/mL.

IA-2A were analyzed in a radioligand-binding assay using a kit analogous to the GADA kit according to the instructions given by the manufacturer (RSR Limited, Cardiff, UK). The RSR kit has been validated in DASP with 68% study sensitivity and 100% study specificity. The CV was 7.7% at level 2.6 Units/mL and 5.8% at level 25.6 Units/ml.

IAA were determined in a non-competitive radioligand-binding assay using 125I-insulin essentially as described [28] and recently modified [29]. The results were expressed in arbitrary units derived from in-house positive and negative standard samples. Intra-assay CV was 6% and inter-assay CV 13%. Our laboratory has been validated in DASP with 20% study sensitivity and 98% study specificity.

A*utoantibodies to the Zinc transporter variants.* The radio-binding assay (RBA) for all three variants, ZnT8R, ZnT8W and ZnT8Q of human ZnT8 (Slc30A8) were performed separately with 5 µL of human sera essentially as described [30]. Duplicate serum samples were incubated over night at 4°C with labeled antigen diluted in antigen buffer. Antibody-bound was separated from free antigen by Protein A-Sepharose. Bound radioactivity was converted into in-house units using a high-titer standard with high ZnT8RA, ZnT8WA or ZnT8QA reactivity. Intra-assay CV for the ZnT8RA was 6%, ZnT8WA 5% and ZnT8QA 4%. Inter-assay CV for ZnT8RA was 7%, ZnT8WA 8% and ZnT8QA 10%. DASP inter-laboratory comparison is in progress.

HLA genotyping HLA-DQB1 and DQA1 genotypes were typed by sequence-specific oligonucleotide probes as described [28, 29, 31] using a DELFIA hybridization assay (Perkin Elmer, Boston, MA, USA). 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 DQA1*0201, 03 and 05 alleles. We compared patient HLA frequencies with 1011 newborn children in the Diabetes Prediction in Skåne study [32, 33].

Statistical methods Statistical analyses were performed using SPSS statistical software (version 17.0; SPSS, Chicago, IL, USA). Differences in proportions between groups were tested using the chi-square test or Fisher´s exact test when appropriate. Odds ratios (OR) with 95% confidence interval (CI) 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.

To measure the agreement between islet autoantibodies, we used the Kappa statistic [34]. A kappa value of 1.0 indicates perfect agreement, whereas a kappa of <0 indicates agreement equivalent to chance

Interpretation of Kappa:

Kappa	Agreement
<0	Less than chance agreement
0.01-0.20	Slight agreement
0.21-0.40	Fair agreement
0.41-0.60	Moderate agreement
0.61-0.80	Substantial agreement
0.81-0.99	Almost perfect agreement

Results

Islet autoantibodies at the time of clinical diagnosis. Among the 686 patients studied at the time of T1D diagnosis, the most common islet autoantibody was ICA (78%) followed by IA-2A (76%), GADA (66%), ZnT8WA (50%), ZnT8RA (50%), IAA (46%) and ZnT8QA (36%) (Table I). Taken all three ZnT8RWQA as one group, 449 (65%) were positive alone or in combination with the other islet autoantibodies. Among the 46 patients with only one islet autoantibody, ZnT8WA was found in 5 (11%), ZnT8RA in 4 (9%) and ZnT8QA in 1 (2%) compared to GADA that was found in 12 (26%), ICA in 10 (22%), IAA in 8 (17%) and IA-2A in 6 (13%), (Table I). These results demonstrate that the three ZnT8RWQA contributed to the diagnostic sensitivity of T1D as 13 (2%) patients without any other islet autoantibody had ZnT8RA, ZnT8WA or ZnT8QA alone. The remaining three patients had autoantibodies recognizing all three ZnT8RWQA. The ZnT8QA was not found among the patients with only two islet autoantibodies and rarely among those with three islet autoantibodies (Table I). One or more of the ZnT8RWQA were present among 70-74% of the patients with either ICA, GADA, IA-2A or IAA (Table I).

The number of islet autoantibodies was next analyzed (Table I). A total of 10% of the patients had all 7 islet autoantibodies, 16% had 6 islet autoantibodies, 17% had 5 islet autoantibodies, 17% had 4 islet autoantibodies, 19% had 3 islet autoantibodies, 9% had 2 islet autoantibodies and 7% had 1 islet autoantibody. It is noted that only 37 (5 %) patients were islet autoantibody-negative. With the inclusion of ZnT8RWQA, the diagnostic sensitivity of islet autoantibodies for T1D increased from 93% to 95%.

Does ZnT8RWQA replace ICA? It was also tested whether ZnT8RWQA would replace ICA as a T1D marker at the time of clinical diagnosis. Disregarding the presence of GADA, IA-2A or IAA alone or in combination, a total of 392 (57%) patients had both ICA and any of the ZnT8RWQA, 141 (21%) patients had only ICA, 57 (8%) had only ZnT8RWQA, and 96 (14%) had neither one. This distribution suggested that disregarding GADA, IA-2A or IAA, an ICA measurement would not be replaceable by a ZnT8RWQA analysis.

Kappa statistics were used to test the degree of agreement between the different islet autoantibody tests (Table II). The autoantibodies against ZnT8R, ZnT8W and ZnT8Q were collapsed into one group referred to as ZnT8RWQA for clarity, as there was no difference when we analyzed the ZnT8RWQA separately. There was moderate agreement between ICA and IA-2A. Fair agreement was observed for ICA – ZnT8RWQA, IA-2A – ZnT8RWQA and ICA – GADA. Only slight agreement was observed for all combinations with IAA (Table II). Furthermore, Kappa statistics confirmed that ICA was in moderate or fair agreement with all islet autoantibody combinations except IAA (data not shown).

Is the diagnostic sensitivity of islet autoantibodies associated with the age at diagnosis? It was tested whether islet autoantibodies were associated with age at diagnosis (Figure 1). The data in Figure 1 demonstrate that IAA was more frequent among children below five years of age 87 of 108 (81%) compared to those who developed T1D above 15 years of age 22 of 84 (26%) ($p<0.0001$). Among the other islet autoantibodies, there was no indication that the frequency was related to the age at diagnosis. The age-dependent diagnostic sensitivity of the ZnT8RWQA taken together as one group did not differ from ICA, GADA or IA-2A (Figure 1). ZnT8WA tended to show a lower diagnostic sensitivity compared to both ZnT8RA and ZnT8QA (data not shown).

Does the number of islet autoantibodies affect age-dependent diagnostic sensitivity? We tested whether the number of islet autoantibodies was related to the age at diagnosis. The data demonstrate that 3-5 islet autoantibodies (the ZnT8RWQA have been collapsed into one group for clarity) predominated regardless of age at diagnosis. In contrast, only two out of 108 (2%) patients below 5 years of age were diagnosed without any islet autoantibody. Diagnosis without any islet autoantibody increased with increasing age at onset (0 – 4 years, 1.9%; 5 – 9 years 4.4%; 10 – 14 years 6.8%; 15 – 19 years, 9.5%; p-value for trend was 0.009).

Autoantibodies and Gender The study included 373 (54%) boys and 313 (46%) girls. Twice as many boys (46; 68%) as girls (22; 32%) were diagnosed with T1D between 1

and 3 years of age (p=0.02). At 4-6 years of age, there were, however, more girls (59%) than boys (p=0.001). As expected, the effect of gender changed again at 13-16 years of age, where 91 boys (61%) were diagnosed compared with 57 girls (p=0.05).
There were more boys (68%; p=0.01) in the group of 72 patients who were positive for all seven autoantibodies. Boys were more often positive for ZnT8QA (60%; p=0.02) and ZnT8WA (58%, p=0.04). GADA was more common among the girls (p=0.004). There was no gender difference for IA-2A, IAA or ICA (data not shown).

HLA and type 1 diabetes. All patients were typed for HLA-DQ alleles known to be associated with T1D and compared with the HLA frequency in controls representing the general population in the Skåne County (Table III). T1D was significantly associated with six different HLA-DQ genotypes, all containing DQA1-B1*03-0302, *05-02, or both (32%). The HLA-DQ genotypes which were significantly associated with T1D, represented 66% of the patients (Table III). DQA1*-B1*03-0302 (40%) was the most common haplotype among the patients compared with 13% of the controls (OR 4.52; 95th CI 3.82-5.35, p<0.0001). DQA1-B1*05-02 was present among 28% of the patients compared with 13% of the controls (OR 2.61; 95th CI 2.19-3.12, p<0.0001). At least one of these two haplotypes was found among 87% of the patients compared to 45% of the controls (OR 6.82, CI 5.85-7.94, p<0.0001).

Is HLA associated with ZnT8 autoantibodies? It was discovered that ZnT8RWQA was associated with both DQA1-B1*X-0604 (p=0.0005) and DQA1-B1*X-04 (p=0.04). When analyzing the individual ZnT8RWQA DQA1-B1*X-0604 was associated with both ZnT8RA (p=0.002) and ZnT8WA (p=0.01) but not with ZnT8QA (p=0.07).
We confirmed that DQA1-B1*03-0302 was associated with ICA (p=0.005), IA-2A (p<0.0001), and IAA (p=0.003). We also confirmed that DQA1-B1*05-02 was associated with GADA (p=0.03) but was negatively associated with IA-2A (p<0.0001). Additionally DQA1-B1*X-0604 was associated with GADA (p=0.03) and IAA (p=0.03) and DQA1-B1*X-04 was associated with ICA (p=0.04).

As the Kappa statistics showed an agreement between certain pairs of islet autoantibodies (Table II), we next tested whether pairs of islet autoantibodies were in agreement and whether the patient had DQA1-B1*03-0302 or DQA1-B1*05-02 alone or if both were absent (data not shown). Taken together, the Kappa statistics failed to detect an association between HLA haplotypes and different patterns of agreement between pairs of islet autoantibodies.

Is HLA associated with the number of islet autoantibodies and age at diagnosis? It was observed that the number of islet autoantibodies (2-5 autoantibodies) was significantly associated with DQA1-B1*03-0302 (p-value for trend was <0.001) but not with DQA1-B1*05-02 (p-value for trend was 0.20) (Table IV). The difference between DQA1-B1*03-0302 and DQA1-B1*05-02 in predicting the risk for multiple islet autoantibodies at diagnosis allowed us to analyze the frequency of DQA1-B1*03-0302 in relation to the age at diagnosis. DQA1-B1*03-0302 varied between 48 and 85% (mean 70%). DQA1-B1*05-02, on the other hand, showed the highest frequency among the children younger than 5 years of age (65-70%) but decreased thereafter to reach 30% at 15 years of age (mean 49%) (p<0.0001). Similarly, the high risk DQA1-B1*05-02/03-0302 genotype decreased from 56% at two years of age to 19% at 16 years of age (p-value for trend <0.001). T1D in the absence of both DQA1-B1*03-0302 and DQA1-B1*05-02 increased from 0% at 1 year of age to 27% at 17 years of age (p-value for trend <0.001). The equal representation of DQA1-B1*03-0302 at each age of diagnosis would be consistent with the absence of an apparent association between the number of islet autoantibodies and age at diagnosis (data not shown).

Discussion

Our study revealed that analyses of the three ZnT8RWQ autoantibody variants reduced the frequency of patients without any islet autoantibody from 7.5% to 5.4% - a reduction by 28%. This reduction, comparable to 26% in a recent study [20], was observed despite that all patients were also analyzed for ICA. The group with ICA only represented 1.5% of the patients. Furthermore, analyses of the ZnT8RWQA together with all the other

autoantibodies showed that the youngest children had as high diagnostic sensitivity for multiple autoantibodies as older children. Twice as many boys as girls were diagnosed with T1D below 4 years of age. Multiple islet autoantibody testing may therefore detect T1D in very young children who otherwise may be regarded as having monogenic diabetes.

Mechanistically, we observed that multiple autoantibodies were associated with high-risk HLA. This is important as multiple autoantibodies represent the best predictor for the clinical onset of T1D [35, 36]. We made the interesting observation that DQA1-B1*X-0604 increases the risk for ZnT8RA and ZnT8WA, but not for ZnT8QA, as DQA1-B1*X-0604 only confers risk together with DQA1-B1*03-0302. This observation points at the importance of HLA for the development of immune responses to islet autoantigens that eventually result in immune mediated beta-cell killing. We confirmed that GADA is associated with DQA1-B1*05-02, IAA with DQA1-B1*03-0302 and IA-2 with DQA1-B1*03-0302 [11, 13, 36, 37] and it is of interest that the ZnT8 autoantibodies were not associated with the two most common T1D HLA haplotypes.

The Skåne study represents a large cohort of children diagnosed with T1D during 9 years within the most Southern part of Sweden representing about 1.2 million inhabitants. The representative group of 686 children at the ages 1-18 years therefore provides novel information on the presence of multiple autoantibodies, most importantly, the three ZnT8RWQA variants at the same time as we retained ICA in our analyses. This study was possible because blood was taken from the majority of the children at the time of clinical onset between 1996 and 2005. We had enough blood samples to carry out all analyses in 686 children and adolescents. This study shows that 649 of 686 (95%) children were islet autoantibody positive at diagnosis. The most common autoantibody was still ICA (78%), which is consistent with studies in Finnish children followed until the diagnosis of diabetes [6]. Among the islet autoantibodies the ranking order of IA-2A, GADA and IAA was consistent with some [13, 38] but to the best of our knowledge ranking including the ZnT8RWQA at the time of diagnosis of children is yet to be published. Including ZnT8RWQA, the ranking order of islet autoantibodies at diagnosis

of childhood diabetes would be ICA, IA-2A, GADA, ZnT8RWQA and IAA. Taken the three variants of ZnT8RWQA together, our frequency of 65% was comparable to previous reports in adults [20]. Our frequency of 65% may be explained by the fact that we analyzed all three ZnT8RWQA and did not include patients with suspected MODY or type 2 diabetes.

Using all the autoantibodies tests in our children, only 5.4 % therefore remained islet autoantibody negative. It is possible that some of the remaining 37 patients did not have T1D. However, some of the islet autoantibody-negative children may have had an islet autoantibody in the cord blood [39, 40]. Specifically, we previously found that patients with islet autoantibodies in their cord blood may be autoantibody negative at diagnosis [40]. It cannot be excluded that some of these patients may have autoantibodies to less frequent autoantigens [41]. Furthermore, our observation that ICA alone may represent 1.5% of the patients indicates that the search for additional autoantigens should continue.

As many as 108 children were below 5 years of age. Our data suggest that all three ZnT8RA, ZnT8WA, and ZnT8QA were as common in the young children as in the older children. It is noted that the ZnT8RWQA was almost as common as IAA in children younger than 5 years of age at diagnosis. The observation that ZnT8RWQA tends to pair with ICA, IA-2A and less well with GADA but not with IAA suggests that the three ZnT8RWQ variant autoantibodies should be analyzed also in studies of very young children at risk for T1D.

By adding the ZnT8 autoantibodies to our autoantibody analyses, it was possible to conclude that age at diagnosis was not associated with the number of islet autoantibodies. Developing T1D with multiple autoantibodies was as common in the very young children as in the teenager group. The well-known decrease in IAA frequency by age may explain that the relative contribution of islet autoantibody-negative children was highest in the teenager group. It cannot be excluded that the teenager T1D patients have had IAA but that these autoantibodies disappeared before the clinical onset. This is consistent with ongoing longitudinal studies of children at risk that any of the islet autoantoibodies may

appear to reach significant titers which drop to negativity. Therefore, the number of autoantibodies at the time of clinical onset may not reflect risk during follow-up, as both the DPT-1 [42] and ENDIT [43] studies did not record the number of autoantibodies at the actual diagnosis but recorded the number of autoantibodies that the subject has had during the follow-up although this is not clearly specified [42, 43]. IAA would be the autoantibody that seems to disappear most often. It is noted that we found that all seven islet autoantibodies were present as often in the youngest children (13 %) as in the oldest (8 %).

In this study we observed that the ZnT8WA and ZnT8RA, but not ZnT8QA, were associated with DQA1-B1*X-0604. This observation is of particular interest as this haplotype alone does not confer risk for T1D but only in the presence of DQA1-B1*03-0302. Further studies also in non-diabetic individuals will be necessary to determine whether the association between ZnT8RWQA and 0604 may be explained by the presentation of the ZnT8 antigen on 0604 HLA heterodimers. The possible importance of HLA class II heterodimers to initiate the immune response to islet autoantigens is also reflected by our observation that 49% of the patients without islet autoantibodies had neutral and low risk HLA.

It has been suggested that some islet autoantibody combinations are more frequent than others. We used Kappa statistics because it takes into account that observations can agree or disagree simply by chance [34]. The observation that IAA only showed a slight agreement with any of the other islet autoantibodies underscores the importance of measuring IAA. Taking HLA into consideration did not improve the agreement between IAA and the other islet autoantibodies.

In conclusion, this study revealed that (1) ZnT8RWQA was common in newly diagnosed T1D independent of age; (2) the youngest children had as high diagnostic sensitivity for the ZnT8 autoantibodies as well as for multiple autoantibodies as the older children; (3) DQA1-B1*X-0604 increased the risk for ZnT8RA and ZnT8WA; and (4) agreement between autoantibody pairs was common for all combinations except IAA. These results

are important in classification of diabetes as some children have only one of the three ZnT8RWQA at diagnosis. The ZnT8RWQA may also be important for prediction of childhood T1D and to randomize subjects in prevention and intervention clinical trials.

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Tables

Table I. Islet autoantibodies at diagnosis of type 1 diabetes in 686 children.

	ICA	GADA	IA-2A	IAA	ZnT8RA	ZnT8WA	ZnT8QA	No of Patients
7 Antibodies	+	+	+	+	+	+	+	72
6 Antibodies	+	+	+	-	+	+	+	57
	+	-	+	+	+	+	+	23
	+	+	+	+	+	+	-	7
	+	+	+	+	+	-	+	7
	+	+	-	+	+	+	+	7
	+	+	+	+	-	+	+	5
	-	+	+	+	+	+	+	1
5 Antibodies	+	-	+	-	+	+	+	28
	+	+	+	+	+	-	-	24
	+	+	+	+	-	+	-	24
	+	+	+	-	+	+	-	12
	+	+	+	-	-	+	+	8
	+	+	-	-	+	+	+	5
	+	-	+	+	+	+	-	5
	+	+	+	-	+	-	+	3
	-	+	-	+	+	+	+	2
	+	+	+	+	-	-	+	1
	-	+	+	-	+	+	+	1
	-	+	+	+	+	+	-	1
	+	-	-	+	+	+	+	1
	+	+	-	+	+	+	-	1
	+	+	-	+	+	-	+	1
	+	-	+	+	+	-	+	1
	+	-	+	+	-	+	+	1
4 Antibodies	+	+	+	+	-	-	-	29

	+	+	+	-	+	-	-	22
	+	+	+	-	-	+	-	17
	+	-	+	+	-	+	-	9
	+	-	+	+	+	-	-	8
	-	-	-	+	+	+	+	4
	+	-	+	-	+	+	-	3
	+	-	+	-	-	+	+	3
	+	+	-	+	+	-	-	2
	+	+	-	+	-	+	-	2
	+	+	-	-	-	+	+	2
	-	+	-	-	+	+	+	2
	-	-	+	-	+	+	+	2
	+	-	-	-	+	+	+	2
	-	+	+	+	+	-	-	1
	-	+	+	+	-	+	-	1
	+	+	-	+	-	-	+	1
	-	+	-	+	+	+	-	1
	+	+	-	-	+	+	-	1
	-	-	+	+	+	+	-	1
	+	+	+	-	-	-	+	1
3								
Antibodies	+	+	+	-	-	-	-	48
	+	-	+	+	-	-	-	17
	+	+	-	+	-	-	-	13
	+	-	+	-	-	+	-	12
	-	+	+	+	-	-	-	7
	+	+	-	-	+	-	-	5
	+	-	+	-	+	-	-	5
	+	+	-	-	-	+	-	3
	-	-	-	-	+	+	+	3
	-	+	+	-	+	-	-	2
	-	+	-	+	+	-	-	2
	-	-	+	+	-	+	-	2
	+	-	-	+	+	-	-	2
	-	+	+	-	-	+	-	1
	-	+	+	-	-	-	+	1
	-	+	-	+	-	+	-	1

	-	+	-	-	-	+	+	1
	-	-	+	+	+	-	-	1
	-	-	+	-	-	+	+	1
	-	-	-	+	+	-	+	1
2								
Antibodies	-	+	+	-	-	-	-	14
	+	-	+	-	-	-	-	13
	+	+	-	-	-	-	-	8
	-	-	+	+	-	-	-	6
	-	+	-	+	-	-	-	5
	-	+	-	-	+	-	-	3
	-	+	-	-	-	+	-	3
	-	-	+	-	-	+	-	3
	+	-	-	+	-	-	-	3
	-	-	-	+	+	-	-	2
	-	-	+	-	+	-	-	1
	-	-	-	+	-	+	-	1
	+	-	-	-	+	-	-	1
1 Antibody	-	+	-	-	-	-	-	12
	+	-	-	-	-	-	-	10
	-	-	-	+	-	-	-	8
	-	-	+	-	-	-	-	6
	-	-	-	-	-	+	-	5
	-	-	-	-	+	-	-	4
	-	-	-	-	-	-	+	1
0 Antibody	-	-	-	-	-	-	-	37
Total	533	450	519	316	341	345	249	686
%	78	66	76	46	50	50	36	

Table II. Agreement of pairs of islet autoantibodies to mark the islet autoimmunity in newly diagnosed type 1 diabetes children.

Ab1 – Ab2	Rank[a]	Kappa (SE)
ICA – IA-2A	1	0.51 (0.04)
ICA – ZnT8RWQA	2	0.31 (0.04)
IA-2A – ZnT8RWQA	3	0.28 (0.04)
ICA – GADA	4	0.25 (0.04)
GADA – IA-2A	5	0.20 (0.04)
GADA – ZnT8RWQA	6	0.13 (0.04)
IAA – ICA	7	0.12 (0.03)
IAA – ZnT8RWQA	8	0.12 (0.03)
IAA – IA-2A	9	0.09 (0.03)
IAA – GADA	10	0.01 (0.04)

Note: a = ranked by agreement to mark autoimmunity (kappa).

Interpretation of Kappa:

Kappa	Agreement
0.81-0.99	Almost perfect agreement¸
0.61-0.80	Substantial agreement
0.41-0.60	Moderate agreement
0.21-0.40	Fair agreement
0.01-0.20	Slight agreement
<0	Less than chance agreement

Table III. HLA-DQ genotypes and number of islet autoantibodies at diagnosis of type 1 diabetes in 686 children.

HLA-DQA1-B1	HLA-DQA2-B2	Number of islet autoantibodiies								Total	Skåne%	Control n	Control %	OR	CI	P
		0	1	2	3	4	5	6	7							
05-02	03-0302	6	16	17	47	44	40	29	22	221	32.2	33	3.3	14.1	9.6-20.6	<0.0001
X-0302	X-0302	3	3	8	14	9	9	10	16	72	10.5	15	1.5	7.8	4.4-13.7	<0.0001
X-0302	X-0501	3	1	4	11	6	15	9	3	52	7.6	29	2.9	2.8	1.7-4.4	<0.0001
05-02	05-02	3	7	2	7	2	8	11	2	42	6.1	20	2.0	3.2	1.9-5.6	<0.0001
X-0302	X-0604	2	1	4	7	6	7	10	3	40	5.8	11	1.1	5.6	2.9-11.1	<0.0001
X-0302	X-04	1	4	2	4	8	2	1	5	27	3.9	15	1.5	2.7	1.4-5.2	0.002
X-0301/0303	X-0302-0304			4	4	4	4	4	5	25	3.6	41	4.1			
05-02	X-0501	1	1	3	6	4	1	3		19	2.8	25	2.5			
X-0302	X-0603	1	1	1	3	3	4	4	1	18	2.6	24	2.4			
05-02	X-0604	2	1	1	2	4	1		2	13	1.9	12	1.2			
0201-02	03-0302		1		3	2	2	1	2	11	1.6	16	1.6			
X-0301	X-0501			1		2	4	2		9	1.3	39	3.9			
05-02	X-0301	1	1	1		2	3	1		9	1.3	24	2.4			
05-02	0201-02		2	1	1	1		3		8	1.2	19	1.9			
X-0301	X-0301/0303	1	1		2	1	2	1		8	1.2	47	4.7			
X-0501	X-0501				2	3	1	1		7	1.0	14	1.4			
X-0301	X-0604			1		1	4	1		7	1.0	10	1.0			
X-04	X-0501			1	1	1	2	1	1	7	1.0	8	0.8			
03-02	03-0302	1			1		2	1	1	6	0.9	2	0.2			
0201-02	X-0501			3	1			1	1	6	0.9	20	2.0			
05-02	X-04	1		1	1	2	1			6	0.9	7	0.7			
X-0501	X-0604			2	1		1		1	5	0.7	9	0.9			
05-02	03-0303				3	2				5	0.7	5	0.5			
0201-02	X-04		1	1		1	1		1	5	0.7	3	0.3			
X-0302	X-0303						1	2	2	5	0.7	11	1.1			
05-02	X-0603	1		1			1	1		4	0.6	18	1.8			
05-02	X-0502/0504	1	1			1				3	0.4	4	0.4			
0201-02	03-0301			1	1				1	3	0.4	5	0.5			
X-0501	X-0503	1				1		1		3	0.4	3	0.3			
05-02	X-0609				1			1		2	0.3	2	0.2			
05-02	X-0601	1			1					2	0.3	2	0.2			
X-0301	X-0503	1	1							2	0.3	0	0.0			
X-0302	X-0502/0504				1		1			2	0.3	5	0.5			
05-02	03-0304							1	1	2	0.3	0	0.0			
05-02	X-0602	1				1				2	0.3	38	3.8			
0201-02	X-0303							2		2	0.3	11	1.1			
X-0501	X-0502/0504	1						1		2	0.3	4	0.4			
X-04	X-0502/0504			1						1	0.1	1	0.1			
X-0302	X-0609			1						1	0.1	4	0.4			
0201-02	X-0601			1						1	0.1	1	0.1			

X-04	X-0604		1							1	0.1	0	0.0
0201-02	X-0503	1								1	0.1	6	0.6
0201-02	X-0502/04								1	1	0.1	2	0.2
0201-02	X-0604							1		1	0.1	6	0.6
03-02	03-0301							1		1	0.1	0	0.0
X-0301	X-04	1								1	0.1	8	0.8
X-0304	X-0604					1				1	0.1	0	0.0
X-0304	X-0501				1					1	0.1	0	0.0
05-02	03-02					1				1	0.1	0	0.0
X-0303	X-0604					1				1	0.1	2	0.2
X-0501	X-0603				1					1	0.1	12	1.2
0201-02	05-0301				1					1	0.1	17	1.7
X-0303	X-0602	1								1	0.1	10	1.0
X-0302	X-0602						1			1	0.1	4	4.0
X-0301	X-0603		1							1	0.1	26	2.6
X-0304	X-0602						1			1	0.1	1	0.1
X-0604	X-0604							1		1	0.1	5	0.5
X-0303	X-04								1	1	0.1	4	0.4
03-02	X-0501							1		1	0.1	0	0.0
03-02	X-04		1							1	0.1	0	0.0
05-02	03-X	1								1	0.1	0	0.0
Total		37	46	63	128	114	119	107	72	686			

Table IV. Number of islet autoantibodies associated with the presence of either DQA1-B1*03-0302 or DQA1-B1*05-02 haplotype at diagnosis of type 1 diabetes in 686 children.

Number of Islet Abs	No. patients	Presence of HLA DQA1-B1*03-0302			
		(%)	OR	95%CI	p-value
None	37	45.9	1.00	Ref	
One	49	57.1	1.52	0.65 – 3.55	
Two	76	64.5	2.38	1.07 – 5.27	
Three	170	73.5	3.40	1.65 – 7.03	
Four	214	71.0	3.03	1.50 – 6.13	
Five	140	78.6	4.53	2.13 – 9.65	<0.001
Number of Islet Abs	**No. patients**	**Presence of HLA DQA1-B1*05-02**			
		(%)	**OR**	**95%CI**	**p-value**
None	37	48.6	1.00	Ref	
One	49	63.3	1.72	0.73 – 4.08	
Two	76	43.4	0.77	0.35 – 1.68	
Three	170	52.4	1.09	0.54 – 2.20	
Four	214	51.4	1.06	0.53 – 2.11	
Five	140	41.4	0.71	0.35 – 1.45	0.20

Notes: Islet Abs is islet autoantibodies and Ref is reference group. The p-values for trend are shown.

Legend To Figure

Figure 1. Frequency (%) of islet autoantibodies in relation to age in the entire group of 686 newly diagnosed type 1 diabetes patients. The islet autoantibodies analyzed were GADA (♦), IA-2A (■), IAA (▲), ICA (x), and ZnT8RWQA (●).

Figure

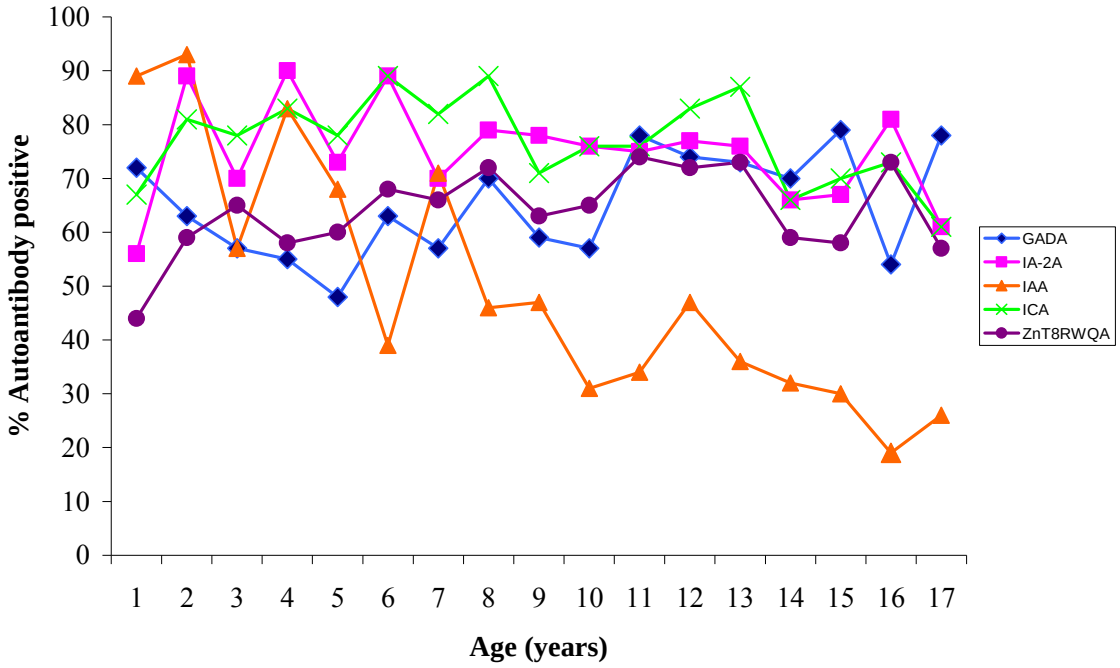

Figure 1