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Islet autoantibodies and residual beta cell function in type 1 diabetes children followed for 3-6 years.

JS Sorensen[1], F Vaziri-Sani[2], M Maziarz[3], K Kristensen[1], A Ellerman[4], N Breslow[3], Å Lernmark[2], F Pociot[5], C. Brorsson[5], NH Birkebaek[1], on behalf of the Danish Study Group for Childhood Diabetes.

1. Department of Pediatrics, Aarhus University Hospital, Skejby, DK-8200 Aarhus N, Denmark.
2. Department of Clinical Sciences, Lund University/CRC, Skåne University Hospital SUS, SE-20502 Malmö, Sweden.
3. Department of Biostatistics, University of Washington, Seattle, WA 98195.
4. Department of Pediatrics, Holbaek Hospital, DK-4300 Holbæk, Denmark.
5. Glostrup Research Institute, Department of Clinical Experimental Research, Ndr. Ringvej 69, DK- 2600 Glostrup, Denmark.

Correspondence to

NH Birkebaek

Department of Pediatrics

Aarhus University Hospital, Skejby

DK-8200 Aarhus

Denmark N

Phone: +4589496713

Fax: +4589496023

Email: nielbirk@rm.dk

Aims. To test if islet autoantibodies at diagnosis of type 1 diabetes (T1DM) and after 3-6 years with T1D predict residual beta-cell function (RBF) after 3-6 years with T1D.

Methods. T1D children (n=260, median age at diagnosis 9.4, range 0.9–14.7 years) were tested for GAD65, IA-2, ZnT8R, ZnT8W and ZnT8Q autoantibodies (A) at diagnosis, and 3-6 years after diagnosis when also fasting and stimulated RBF were determined.

Results. For every 1-year increase in age at diagnosis of TID, the odds of detectable C-peptide increased 1.21 (1.09, 1.34) times for fasting C-peptide and 1.28 (1.15, 1.42) times for stimulated C-peptide. Based on a linear model for subjects with no change in IA-2A levels, the odds of detectable C-peptide were 35% higher than for subjects whose IA-2A levels decreased by half (OR=1.35 (1.09, 1.67), p=0.006); similarly for ZnT8WA (OR=1.39 (1.09, 1.77), p=0.008) and ZnT8QA (OR=1.55 (1.06, 2.26) p=0.024). Such relationship was not detected for GADA or ZnT8RA. All OR adjusted for confounders.

Conclusions. Age at diagnosis with T1D was the major predictor of detectable C-peptide 3-6 years post diagnosis. Decreases in IA-2A, and possibly ZnT8A, levels between diagnosis and post diagnosis were associated with a reduction in RBF post diagnosis.

Keywords: GAD65 autoantibodies, IA-2 autoantibodies, ZnT8 autoantibodies, residual beta cell function, age at diagnosis.

Introduction.

Type 1 diabetes (T1D) is an autoimmune disease with selective destruction of the pancreatic islet beta cells. During the autoimmune process, autoantibodies against beta cell autoantigens are generated. Autoantibodies against the 65 kDa isoform of glutamate decarboxylase (GADA), insulin (IAA), and the insulinoma antigen 2, IA-2 (IA-2A) have been well characterized and provide predictive markers for T1D (1;2). An additional islet cell autoantigen - the zinc transporter 8 (ZnT8) has recently been described (3). The ZnT8 autoantigen has a unique C-terminal end epitope at amino acid position 325 distinguished by specific autoantibodies against either Arg (R), Trp (W) or Gln (Q), alone or in combination. Zn T8 autoantibodies were reported to be more prevalent against the Arg_{325} and Trp_{325} than against Gln_{325} (4;5). Autoantibodies against the ZnT8 variants (ZnT8A) may increase the predictive value of islet autoantibodies for T1D (3;6). Further, it has been claimed that ZnT8A may be useful in monitoring residual beta-cell function after diagnosis of T1D (3).

Several studies have related residual beta-cell function (RBF), evaluated by fasting or stimulated C-peptide, to islet autoantibodies after the diagnosis of T1D in adults (7-9). However, RBF after the diagnosis of T1D in relation to islet autoantibodies and HLA genotypes has not been thoroughly examined in children (10;11), and no studies have evaluated the possible effects of IA-2A and ZnT8A on RBF. Furthermore, the increasing incidence of childhood diabetes has been shown to be associated with a reduced contribution of high risk HLA haplotypes (12;13). This might change the distribution of islet autoantibodies and the rate of losing RBF after the diagnosis of T1D especially as GADA, IA-2A and ZnT8A positive patients may account for more than 95% of all children diagnosed with T1D.

The aim of the study was to examine whether autoantibodies against GAD65, IA-2 and all three variants of ZnT8 at amino acid position 325 (R, W and Q) either 1) at diagnosis, 2) after 3-6 years post diagnosis or 3) the change in autoantibody level between diagnosis and 3-6 years post diagnosis would predict RBF at 3-6 years. Further, we explored the associations between HLA-DQB1 diabetes risk alleles, age at diagnosis, diabetes duration and islet autoantibodies with RBF 3-6 years post diagnosis.

Patients and methods.

Patients. A total of 260 children (130 girls) with T1D for 3-6 years were studied (Table 1). The median age at diagnosis was 9.4 years (range 0.9–14.7 years) with no difference between boys and girls. All patients were registered in the Danish Registry for Childhood Diabetes. Serum samples from diagnosis were stored at -80^{o} C in the repository of the Danish Registry for Childhood Diabetes. At follow-up 3-6 years after diagnosis, blood samples were taken before breakfast and morning insulin. If patients were treated with continuous subcutaneous insulin injections, the basal insulin was continued with a 25% dose reduction during the last eight hours. Fasting blood glucose (BG) was determined and serum for C-peptide and islet autoantibodies was obtained just before the ingestion of a liquid meal [boost drink, formerly known as Sustacal (8 fluid ounces containing 33 g carbohydrate, 15 g protein, and 6 g fat, 240 kcal); 6 ml/kg (maximal 360 ml); Novartis Medical Health, Inc., Minneapolis, MN, www.boost.com]. BG was measured and serum for stimulated C-peptide was taken 90 minutes after the ingestion of the drink (14). The C-peptide level was used as a surrogate marker for the RBF (14). Fasting blood samples from healthy school children (n=80), without any relation to the registry patients, constituted a control group for the islet autoantibody assays.

C-peptide. Blood samples for serum C-peptide test were centrifuged for 10 min at 20^{o}C and stored at -80^{o}C until analysis. C-peptide was detected using a fast phase-, fluoroimmunometric sandwich assay on a 1235 AutoDELPHIA^{TM} (Perkin Elmer-Wallach, Turku, Finland), EN/ISO 15 189. The detection limit was 0.01 nmol/L and the measuring range 0.01-6 nmol/L. The intra-assay coefficient of variation was 4.3 % (0.1 nmol/L), 4.7% (1 nmol/L) and 3.6% (2.5 nmol/l), respectively. The inter-assay coefficient of variation was: 5.0 % (0.1 nmol/L), 4.5% (1 nmol/L) and 2.7% (2.5 nmol/l), respectively. C-peptide results were dichotomized into 'detectable' and 'non-detectable', respectively.

Autoantibody assays. Autoantibodies against GAD65, IA-2, ZnT8R, ZnT8W and ZnT8Q were measured as previously described in detail (15-20). The diabetes autoantibody standardization program (DASP) performances were as follows: The intra-assay coefficient of variation for duplicates was 7% for GAD65A, 11% for IA-2A, 8% for ZnT8RA, 8% for ZnT8WA and 9% for ZnT8QA. The inter-assay coefficient of variation was 11% for GADA, 8% for IA-2A, 10% for

ZnT8RA, 10% for ZnT8WA and 5% for ZnT8QA. In the DASP 2009 workshop our laboratory was among the top ranking laboratories for GAD65A in workshop sensitivity (68%) and specificity (99%) and the top ranking laboratory for IA-2A in workshop sensitivity (60%) and specificity (99%). In the recent DASP workshop on ZnT8A (21), our laboratory had 38% workshop sensitivity and 100% specificity for ZnT8QA, 52% and 100%, respectively for ZnT8RA and 50% and 100%, respectively for ZnT8WA.

The 98.5th percentile in 98 healthy school children was used as the cut-off level for positivity in all five islet autoantibody tests. Sera from healthy children and patients were analyzed in parallel. The thresholds were: 46 U/ml for GADA, 2 U/ml for IA-2A 6 U/ml for ZnT8RA, 38 U/ml for ZnT8WA and 43 U/ml for ZnT8QA .

HLA genotyping. HLA-typing (DQB1) was performed by DELFIA (PerkinElmer, Turku, Finland) method following manufacturer´s instructions. This method combines polymerase chain reaction using sequence specific primers ([gcatgtgctacttcaccaacg] (forward primer), [Biotin-ccttctggctgttccagtact] reverse primer)) time-resolved fluorometry, and use of lanthanide-labeled, short allele-specific oligonucleotide probes (basic assay: *301, *302, *602, *603, *02, control) in a solution hybridization reaction (22). The HLA-DQB1 types were classified as: very high risk genotypes: 302/02, high risk genetypes: 302/302 and 302/X where X is any allele except 02, neutral risk genotypes: 02/02 and 02/X where X is any allele except 0302 and all others as low risk genotypes: 604/x, 604/301, 603/304, 602/304, 602/301, 603/x, 602/x, 304/x, 301/x.

Statistical analysis.

Unless stated otherwise, the outcome was detectable stimulated C-peptide at 3-6 years post diagnosis, referred to simply as 'C-peptide'. Autoantibody levels for GADA, IA-2A, ZnT8RA, ZnT8WA and ZnT8QA at diagnosis and at 3-6 years post diagnosis were dichotomized as 'present' or 'absent' according to cut-off limits defined above, or $\log_2$ transformed. A measure of change in antibody levels over time on study was defined as $\log_2$ of the ratio of autoantibody levels at 3-6 years and at diagnosis.

The association between C-peptide and islet autoantibodies was modeled using logistic regression with C-peptide, $\log_2$ of the ratio of autoantibody levels at 3-6 years and at diagnosis (change in autoantibody level over time on study), adjusted for ($\log_2$) baseline islet autoantibody levels in the

main analysis, as well as age at diagnosis, HLA risk group, gender and diabetes duration in the adjusted analysis. ZnT8 autoantibodies were highly correlated; to avoid co-linearity, we modeled GADA, IA-2A and one of the ZnT8 autoantibodies at a time. This resulted in three unadjusted and three adjusted analyses.

We estimated the association between C-peptide and either age at diagnosis using logistic regression with age at diagnosis as the main effect, adjusted for HLA risk, diabetes duration, sex, baseline levels and changes in GADA, IA-2A and one of the ZnT8 autoantibodies (3 adjusted models). No adjustment for multiple comparisons was made, instead we present the results of all of the analyses.

Exploratory analysis. Relationships between age at diagnosis and number of islet autoantibodies, as well as HLA genotype were plotted.

Sensitivity analysis. A Wilcoxon rank sum test was used to test whether C-peptide was associated with any islet autoantibody, testing one autoantibody at a time.

All analyses were performed in R 2.9.2 (www.r-project.org).

Ethics

The study was performed in accordance with the ethical principles of the Declaration of Helsinki II. The National Ethics Committee approved the study protocol. All children and parents gave written informed consent.

Results

GADA, IA-2A and ZnT8A. The percentage of autoantibody positive patients decreased between diagnosis and 3-6 years from 64% to 40% for GADA, 85% to 78% for IA-2A, 66% to 48% for ZnT8RA, 56% to 36% for ZnT8WA and 44% to 33% for ZnT8QA. 71% of patients were positive for at least one ZnT8 autoantibody at diagnosis, and 55% were positive for at least one ZnT8 autoantibody at 3-6 years post diagnosis. A total of 76/260 (29%) patients were positive for all five autoantibodies at diagnosis decreasing to 34 (13%) after 3-6 years (Figure 1). Only nine (3%) of the patients were autoantibody negative at diagnosis, increasing to 26 (10%) after 3-6 years. The patients negative for all five autoantibodies 3-6 years post diagnosis tended to be younger at onset (Figure 1, right panels). The loss of islet autoantibodies after 3-6 years primarily affected the children with 3-5 autoantibodies at diagnosis, but all were more likely to lose an autoantibody than to gain one over 3-6 years (Figure 1, left panel).

RBF after 3-6 years of T1D. After 3-6 years of T1D, a total of 137 children had stimulated C-peptide below and 123 patients above 0.01nmol/L. Age at diagnosis was strongly associated with a detectable level of both fasting and stimulated C-peptide (Table 1). The estimated odds (95% CI) of having a detectable fasting C peptide at 3-6 years was 1.21 (1.09, 1.34) p=0.0005, for subjects one year older at diagnosis with TID compared to the reference group after adjusting for HLA risk group, gender, diabetes duration and for baseline levels as well as changes over time for GADA, IA-2A and ZnT8RA. Similarly, the estimated odds (95% CI) of having a detectable stimulated C peptide at 3-6 years was 1.28 (1.15, 1.42) p<0.0001 for subjects one year older at diagnosis with TID compared to the reference group, after adjusting for the same factors as for fasting C-peptide. The complete analyses, unadjusted as well as adjusted, for all three models are shown in Appendix Table 1.

GADA, IA-2A and ZnT8A in relation to RBF at 3-6 years of T1D. Levels of islet autoantibodies at diagnosis were not found to be associated with C-peptide levels 3-6 years post diagnosis (Appendix Table 2). However, on average, subjects experienced a 2.35-fold (interquartile range 1.19 - 3.61) decrease in the level of IA-2A from diagnosis to 3-6 years post diagnosis. Based on a linear model, the odds of maintaining a detectable level of C-peptide post diagnosis was higher (OR 1.35 (1.09, 1.67); p=0.006) for subjects whose IA-2A level was unchanged between diagnosis and 3-6 years post diagnosis compared to those whose level of IA-2A decreased by half over that

same period. This effect of unchanged IA-2A levels was observed after adjusting for baseline GADA, IA-2A and ZnT8QA levels, age at diagnosis, HLA risk, gender, diabetes duration and changes over time in the levels of GADA and ZnT8Q (Table 2). The corresponding estimates associated with the change in IA-2A level over time were similar in the analyses involving ZnT8RA and ZnT8WA (Table 2).

The odds of maintaining a detectable level of stimulated C-peptide post diagnosis was not higher (OR 1.07 (0.91, 1.27); p=0.406) for subjects whose level of ZnT8RA was unchanged between diagnosis and 3-6 years post diagnosis compared to subjects whose level of ZnT8RA decreased by half over that time period. These data was obtained after adjusting for baseline GADA, IA-2A and ZnT8RA levels, age at diagnosis, HLA risk, gender, diabetes duration and changes over time in the levels of GADA and IA-2A (Table 2). In contrast, the corresponding estimates for both ZnT8WA (OR 1.39 (1.09, 1.77); p=0.008) and ZnT8WQA (OR 1.55 (1.06, 2.26); p=0.024) indicated that a decrease in these two ZnT8A also decreased the odds of maintaining a detectable level of stimulated C-peptide post diagnosis. Crude odds ratios of having a detectable level of C-peptide post diagnosis for three categories of change in autoantibody level compared to subjects whose levels decreased by more than 85% (or were undetectable at 3-6 years) were summarized in Table 1.

Discussion

This study describes the presence of GADA, IA-2A and all three variants of ZnT8A at diagnosis and after 3-6 years with diabetes stratified for RBF at 3-6 years with diabetes in children. The study population was homogeneous concerning gender and age. Furthermore, there were no gender differences in age at T1D diagnosis. Using all five autoantibody tests, 97% of the patients had one or several islet autoantibodies at diagnosis. This should be kept in mind when comparisons are made to earlier studies where as many as 10-15% of the children might have tested negative for an autoantibody. A first major finding in our study was that age at diagnosis is the strongest predictor for a detectable C-peptide RBF after 3-6 years of T1D. This observation is consistent with previous studies (11;23), and also confirms that many children even 6 years after the diabetes diagnosis may have measurable C-peptide (23). Different studies in children have used different sampling methods for C-peptide determinations such as stimulated C-peptide(11;24), non stimulated fasting C-peptide (23) or random C-peptide (10). We demonstrate significant differences in fasting C-peptide and

stimulated C-peptide, and 24 patients had detectable C-peptide on stimulation while fasting C-peptide was below the detection limit (data not shown). This means that different studies using different blood sampling conditions for C-peptide may provide different results. Using stimulated C-peptide and a highly sensitive assay, as in this study, we believe is the best possible way of estimating RBF (24).

Forty-seven percent of the patients in our study population had a stimulated C-peptide above the detection limit of the assay at 0.01nmol/L, indicating RBF and possible viable beta cells. Residual beta cell of an amount giving rise to a stimulated C-peptide at or just above our detection limit hardly affect the clinical outcome, but may serve as an auto-antigen stimulus for further autoantibody formation, while a beta cell amount giving rise to a stimulated C-peptide of 0.04 nmol/L or more may affect the metabolic control (24). It is therefore of interest that the second major finding in our study was that the change in IA-2 autoantibody level was associated with RBF 3-6 years post diagnosis. We are not aware of any prior publication reporting that change in islet autoantibody levels during follow up may be related to RBF. Jensen et al (8) did not find any association between autoantibodies and beta-cell function in an adult population. However, in that study the detection limit of C-peptide was 0.13nmol/L, and patients categorized with no RBF (C-peptide less than 0.13nmol/L) may have enough beta cell tissue to mount an autoantibody response possibly causing errors of classification. Others measured C-peptide with a detection limit of 0.1nmol/L and showed that the presence of at least two of three autoantibodies (ICA, GADA and IA-2A) at diagnosis predicted no RBF five years after the diagnosis (25). An earlier investigation demonstrated a negative correlation between RBF and ICA (11). Our demonstration that a change in IA-2A autoantibody levels in subjects who were followed prospectively may be related to RBF warrants further investigation not only post diagnosis but also before diagnosis. The change in islet autoantibody levels over time may be used to predict diabetes in children followed prospectively from birth in studies such as TEDDY (26) and DAISY(27).

The autoantibody distribution of GADA and IA-2A at diagnosis was as demonstrated in other studies of T1D in childhood (28;29). The 71% of children who had ZnT8A at diagnosis demonstrate that ZnT8A measurements add a significant number of children who otherwise would have been recorded as autoantibody negative or only single autoantibody positive. No studies on

ZnT8A in children only have been published, but recently in a population of 223 new onset T1D patients with an age range of 1-46 years ZnT8A was detected in 63% (3). Our finding that only nine (3%) of the patients were autoantibody negative at diagnosis and that after 3-6 years this frequency increased to 10% confirms a significant loss of islet autoantibodies including ZnT8A during the first years of T1D (30).

We did not detect any association between HLA-DQB1 alleles and the rate of disappearance of autoantibodies or the number of patients with or without RBF after 3-6 years of T1D. This is in accordance with other studies, which could neither demonstrate an effect of HLA-DQB1 on GADA and IA-2A disappearance rates in adults (8) nor on beta cell function in children.

It is concluded that age at diagnosis with T1D was the major predictor for having detectable levels of C-peptide 3-6 years post diagnosis. Finally, a novel finding was that a decreased reduction in IA-2A level between time at diagnosis and 3-6 years post diagnosis was associated with a higher residual beta-cell function at 3-6 years. This result was driven by subjects whose level of IA-2A decreased by more than one half over time, since there were relatively few subjects whose levels of IA-2A did not change or increased over the 3-6 years. More data would be needed to determine whether the same is true for the ZnT8 autoantibodies. Results of an adjusted analysis based on all data provided some evidence suggesting that a change in the level of ZnT8W, and possibly ZnT8Q, predicted residual beta-cell function at 3-6 years; an unadjusted analysis did not corroborate the initial findings. Hence, though there was some suggestion that a change in the levels of ZnT8W, and possibly ZnT8Q, may be informative about the residual beta-cell function at 3-6 years, at this point we do not have enough evidence to conclude it, nor to dispute it.

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Declaration of Competing Interests

No competing interests.

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Table 1. Number of subjects with type 1 diabetes and the proportion of these with a detectable C-peptide (stimulated C-peptide >0.01nmol/L) in relation to age at diagnosis, gender, HLA risk category, diabetes duration and the change in autoantibody level, along with crude odds ratios of having a detectable level of C-peptide 3-6 years post diagnosis for subjects in a given category compared to those in the reference category.

	Total	**Detectable**	**OR (95% CI)**	**p-value***
	260	**C-peptide %**	**-**	**-**
Age at diagnosis (years)				
(0, 3]	19	10.5	0.06 (0.01, 0.30)	<0.001
(3, 6]	38	31.6	0.23 (0.08, 0.62)	0.001
(6, 9]	63	44.4	0.40 (0.17, 0.92)	0.018
(9, 12]	89	52.8	0.56 (0.25, 1.21)	0.110
(12, 15]	51	66.7	reference	
Gender				
Female	130	47.7	1.03 (0.62, 1.73)	0.901
Male	130	46.9	reference	
HLA risk				
Low (X/X)	42	57.1	1.61 (0.73, 3.59)	0.200
Neutral (02/02, 02/X)	45	37.8	0.74 (0.33, 1.61)	0.403
High (302/302, 302/X)	78	50.0	1.21 (0.64, 2.30)	0.535
Very high (02/302)	95	45.3	reference	
Diabetes duration (years)				
(3, 4]	102	55.9	2.08 (1.04, 4.23)	0.025
(4, 5]	97	44.3	1.31 (0.65, 2.68)	0.411
(5, 6]	61	37.7	reference	
Change in autoantibody level from diagnosis to 3-6 years post diagnosis (post/at diagnosis)				
GADA decrease (%)	**Total**	**% Detected**	**OR (95% CI)**	**p-value**
< 25%	57	59.6	3.48 (1.42, 8.92)	0.003
25-65%	76	50.0	2.37 (1.02, 5.73)	0.029
65-85%	83	45.8	2.00 (0.87, 4.80)	0.076
> 85%	44	29.5	reference	
IA-2A decrease	**Total**	**% Detected**	**OR (95% CI)**	**p-value**
< 25%	39	61.5	2.94 (1.32, 6.77)	0.004
25-65%	40	70.0	4.28 (1.88, 10.29)	<0.001
65-85%	64	46.9	1.63 (0.84, 3.18)	0.119
> 85%	117	35.0	reference	
ZnT8R decrease	**Total**	**% Detected**	**OR (95% CI)**	**p-value**
< 25%	76	50.0	1.51 (0.78, 2.95)	0.210
25-65%	42	64.3	2.70 (1.19, 6.31)	0.009
65-85%	54	42.6	1.12 (0.53, 2.36)	0.740
> 85%	88	39.8	reference	
ZnT8W decrease	**Total**	**% Detected**	**OR (95% CI)**	**p-value**
< 25%	76	47.4	1.96 (0.88, 4.46)	0.072
25-65%	83	60.2	3.28 (1.50, 7.45)	0.001
65-85%	50	42.0	1.58 (0.65, 3.89)	0.268
> 85%	51	31.4	reference	
ZnT8Q decrease	**Total**	**% Detected**	**OR (95% CI)**	**p-value****
< 25%	145	50.3	3.02 (0.98, 11.20)	0.054
25-65%	60	55.0	3.61 (1.07, 14.37)	0.023
65-85%	35	34.3	1.55 (0.40, 6.82)	0.555
> 85%	20	25.0	reference	

***Chi-squared test p-values. **Fisher's exact test p-values.**

Table 2. The estimated odds ratio (OR) and the associated 95% confidence interval of the association between having a detectable level of stimulated C-peptide at 3-6 years post diagnosis for subjects whose antibody level remained unchanged over the time on study, compared to subjects whose antibody level decreased by one half over that time*.

		Unadjusted**					
Autoantibody		**Model 1**		**Model 2**		**Model 3**	
		OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
log2(post/at diagnosis)	**GADA**	1.11 (0.94, 1.31)	0.219	1.08 (0.91, 1.28)	0.364	1.10 (0.93, 1.30)	0.251
	IA-2A	1.48 (1.21, 1.81)	<0.001	1.45 (1.19, 1.76)	<0.001	1.43 (1.17, 1.74)	<0.001
	ZnT8RA	1.03 (0.88, 1.20)	0.733				
	ZnT8WA			1.25 (1.00, 1.55)	0.048		
	ZnT8QA					1.46 (1.03, 2.07)	0.033
		Adjusted* **					
Autoantibody		**Model 1**		**Model 2**		**Model 3**	
		OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
log2(post/at diagnosis)	**GADA**	1.04 (0.87, 1.25)	0.674	1.00 (0.83, 1.21)	0.968	1.04 (0.87, 1.24)	0.688
	IA-2A	1.40 (1.13, 1.73)	0.002	1.36 (1.10, 1.68)	0.004	1.35 (1.09, 1.67)	0.006
	ZnT8RA	1.07 (0.91, 1.27)	0.406				
	ZnT8WA			1.39 (1.09, 1.77)	0.008		
	ZnT8QA					1.55 (1.06, 2.26)	0.024

* Baseline autoantibody levels were adjusted for in both analyses. HLA risk group, age at diagnosis, diabetes duration and gender were adjusted for in the adjusted analysis. To avoid co-linearity issues related to high correlation between the ZnT8 autoantibodies, three separate analyses were performed for GADA, IA-2A and one of the ZnT8 autoantibodies at a time. The p-values reported were not adjusted for multiple comparisons.

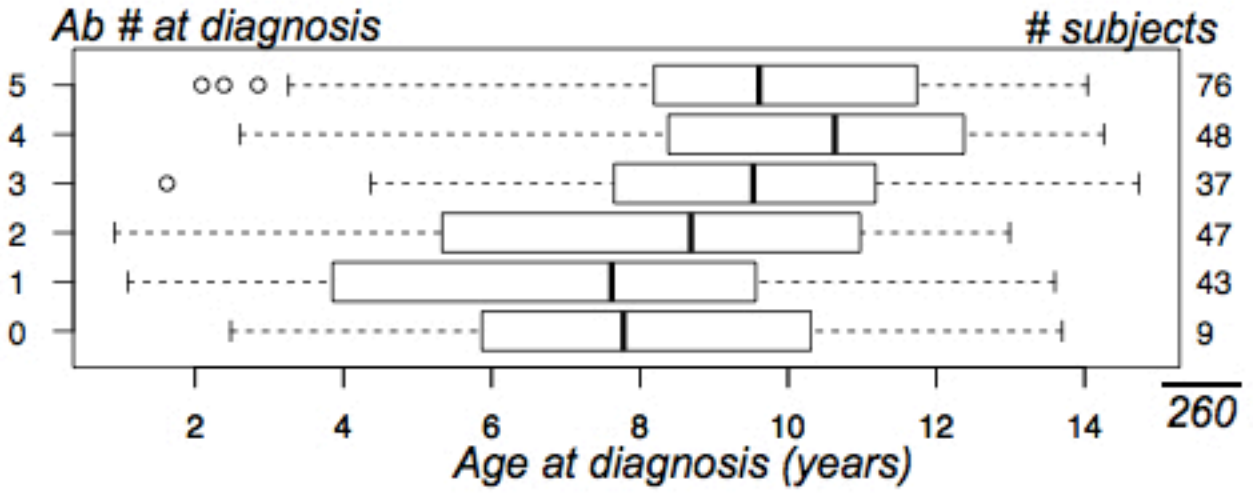
** Adjusted for baseline antibody levels only.

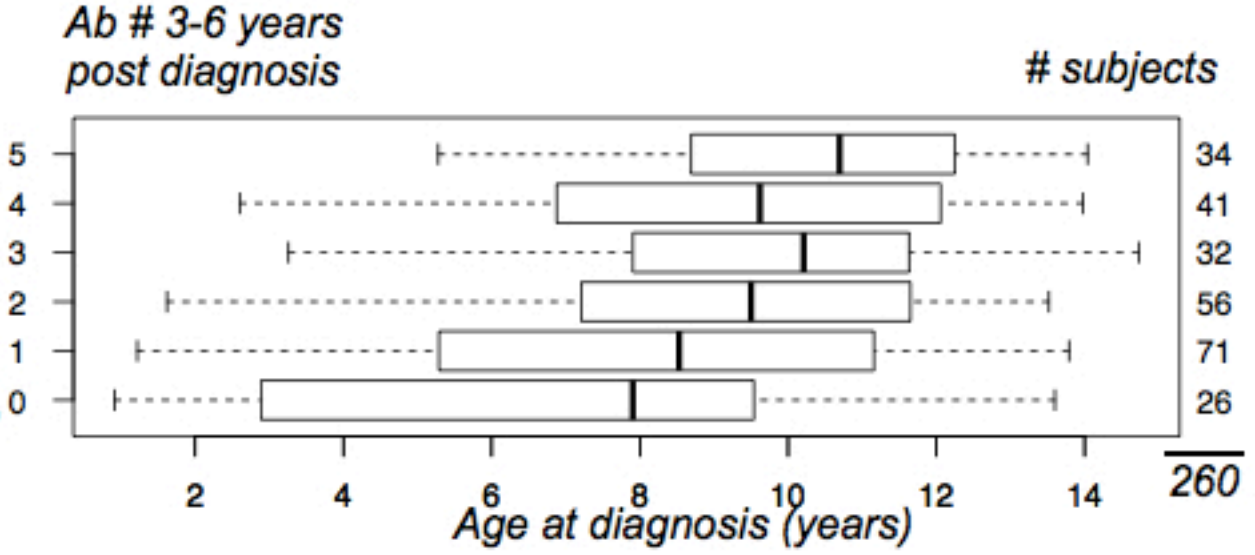
*** Adjusted for baseline antibody levels, HLA risk group, age at diagnosis, diabetes duration and sex.

Distribution of autoantibody counts at diagnosis compared to that at 3-6 years post diagnosis with T1D

		At 3-6 years post diagnosis					
	Ab #	**0**	**1**	**2**	**3**	**4**	**5**
At diagnosis	**0**	3	5	1	0	0	0
	1	13	26	3	0	0	1
	2	7	17	22	0	1	0
	3	2	12	11	10	1	1
	4	1	5	7	14	18	3
	5	0	6	12	8	21	29

Autoantibody positivity thresholds based on the 98.5th percentile of each autoantibody in controls





Appendix

Table 1. The estimated odds ratio (OR) and the associated 95% confidence interval of the association between having a detectable level of fasting (stimulated) C-peptide at 3-6 years post diagnosis for subjects for every year of increase in age at diagnosis with T1D*.

C-peptide	Model	Estimated		
		OR	95% CI	p-value
Fasting	Unadjusted	1.21	(1.11, 1.32)	<0.0001
	Adjusted 1	1.21	(1.09, 1.34)	0.0005
	Adjusted 2	1.22	(1.09, 1.36)	0.0003
	Adjusted 3	1.21	(1.09, 1.34)	0.0004
Stimulated	Unadjusted	1.26	(1.15, 1.37)	<0.0001
	Adjusted 1	1.28	(1.15, 1.42)	<0.0001
	Adjusted 2	1.30	(1.17, 1.45)	<0.0001
	Adjusted 3	1.29	(1.16, 1.43)	<0.0001

*Adjusted 1: HLA risk group, diabetes duration, sex, baseline levels of GADA, IA-2A and ZnT8RA, changes over the study period in GADA, IA-2A and ZnT8RA

Adjusted 2: as in Adjusted 1, with ZnT8WA in place of ZnT8RA

Adjusted 3: as in Adjusted 1, with ZnT8QA in place of ZnT8RA

Table 2. The estimated odds ratio (OR) and the associated 95% confidence interval of the association between having a detectable level of stimulated C-peptide at 3-6 years post diagnosis for subjects whose antibody level at baseline was twice that of the comparison group*.

		Unadjusted**					
Autoantibody		**Model 1**		**Model 2**		**Model 3**	
		OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
log2(baseline)	**GADA**	1.01 (0.93, 1.10)	0.842	1.00 (0.92, 1.09)	0.934	1.00 (0.92, 1.09)	0.963
	IA-2A	1.09 (0.99, 1.20)	0.068	1.08 (0.98, 1.19)	0.140	1.07 (0.98, 1.18)	0.144
	ZnT8RA	1.00 (0.92, 1.09)	0.973				
	ZnT8WA			1.11 (0.97, 1.27)	0.129		
	ZnT8QA					1.19 (0.97, 1.47)	0.100
		Adjusted* **					
Autoantibody		**Model 1**		**Model 2**		**Model 3**	
		OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
log2(baseline)	**GADA**	0.95 (0.86, 1.05)	0.315	0.94 (0.85, 1.04)	0.206	0.94 (0.85, 1.04)	0.242
	IA-2A	1.03 (0.93, 1.15)	0.545	1.01 (0.91, 1.12)	0.837	1.01 (0.91, 1.12)	0.872
	ZnT8RA	0.99 (0.91, 1.08)	0.844				
	ZnT8WA			1.11 (0.96, 1.29)	0.148		
	ZnT8QA					1.19 (0.95, 1.49)	0.128

* Changes in autoantibody levels (log2(post/at diagnosis)) were adjusted for in both analyses. HLA risk group, age at diagnosis, diabetes duration and gender were adjusted for in the adjusted analysis. To avoid co-linearity issues related to high correlation between the ZnT8 autoantibodies, three separate analyses were performed for GADA, IA-2A and one of the ZnT8 autoantibodies at a time. The p-values reported were not adjusted for multiple comparisons.

** Adjusted for changes in antibody levels (log2(post/at diagnosis)) only.

*** Adjusted for changes in antibody levels, HLA risk group, age at diagnosis, diabetes duration and sex.