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Larsson, Helena; Larsson, Christer; Lernmark, Åke

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LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Helena Elding Larsson, MD, PhD[1], Christer Larsson, PhD[2], Åke Lernmark, PhD[1] and the DiAPREV-IT study group.

Baseline heterogeneity in glucose metabolism marks the risk for type 1 diabetes and complicates secondary prevention.

[1] Department of Clinical Sciences, Lund University, Malmö, Sweden.

[2] Department of Laboratory Medicine, Lund University, Lund, Sweden.

Corresponding author:
Helena Elding Larsson, MD, PhD
Department of Clinical Sciences Malmö/ Pediatric Endocrinology, Lund University
Jan Waldenströms gata 35, CRC 60:11
Skåne University Hospital SUS
20502 Malmö, Sweden
+46337676, helena.larsson@med.lu.se

Abstract

Non-diabetic children with multiple islet autoantibodies were recruited to a secondary prevention trial. The objective was to determine the predictive value of baseline 1) HbA1c and metabolic variables derived from intravenous (IvGTT) and oral (OGTT) glucose tolerance tests, 2) insulin resistance and 3) number, type and levels of islet autoantibodies, for progression to type 1 diabetes. Children (n=50, median 5.1 (4-17.9) years) with autoantibodies to glutamate-decarboxylase (GAD65A) and at least one of insulinoma-associated protein 2 (IA-2A), insulin (IAA) or ZnT8 transporter (ZnT8RA, ZnT8WA, ZnT8QA) were screened with IvGTT and OGTT and followed for a minimum of two years. Baseline first phase insulin response (sum of serum-insulin at one and three minutes during IvGTT; FPIR) ≤ 30 µU/mL (HR 4.42 (CI 1.40-14.0) p=0.011) and maximal plasma-glucose ≥ 11.1 mmol/L measured at 30, 60 and/or 90 minutes during OGTT (HR 6.13 (CI 1.79-21.0) p=0.0039) were predictors for progression to diabetes. The combination of FPIR from IvGTT and maximal plasma-glucose during OGTT predicted diabetes in 10/12 children (HR 9.17 (CI 2.0-42.0) p=0.0043). High-level IA-2A, but not number of autoantibodies, correlated to dysglycemia during OGTT (p=0.008) and to progression to type 1 diabetes (HR 4.98 (CI 1.09-22.0) p=0.039). Baseline FPIR, maximal plasma-glucose ≥ 11.1 at 30, 60 or 90 min during OGTT and high-level IA-2A need to be taken into account when randomizing islet autoantibody positive non-diabetic children to secondary prevention.

Clinical trial number: EuCT 2008-007484-16, NCT01122446

word count: abstract 228, main text 3137

Key words: Type 1 diabetes, prediction, intravenous glucose tolerance tests, oral glucose tolerance test, first phase insulin response, dysglycemia, impaired glucose metabolism, IA-2A

Introduction:

Prediction of type 1 diabetes is important when randomizing non-diabetic children with multiple islet autoantibodies into secondary prevention trials. Such trials are complicated by the variability in the progressive autoimmunity, which leads to the destruction of the pancreatic beta cells and eventually results in insulin deficiency.

The autoimmune process, caused by an interaction between environmental factors and genetic predisposition, is often initiated early in life [1,2]. Proposed environmental triggers are viral infections [3-5] infant diet [6,7], early infant growth [8] and insulin resistance [9], among others. A possibility that different environmental events may be associated with diverse patterns in beta cell autoimmunity have been raised [9], indicating that many factors may be involved in the initiation of the autoimmune process.

During the autoimmune process, lasting for months to years before symptoms of insulin deficiency appear, there are realistic opportunities for secondary prevention. The autoimmune beta-cell destruction is reflected by circulating autoantibodies to glutamate decarboxylase (GAD65A), insulinoma-associated protein 2 (IA-2A), any of three epitopes of Zn transporter T8 (ZnT8RA/WA/QA) and insulin (IAA), as dynamic markers of the on-going autoimmune process. Previous studies have revealed that an early seroconversion [2,10] and number of autoantibodies [11,1] are related to risk of type 1 diabetes, but since autoantibodies may appear sequentially during the autoimmune progressive process, the number of autoantibodies at a specific point of time is an uncertain measure.

Although multiple islet autoantibodies in an individual at increased genetic risk may predict type 1 diabetes, the time frame of progression to clinical disease differs. Accurate prediction of time to diabetes is important at inclusion in prevention studies of islet autoantibody positive subjects. Several studies have described a gradually deteriorating glucose metabolism before the diagnosis of diabetes, with increasing HbA1c [12] and 120-minute plasma-glucose in an oral glucose tolerance test (OGTT) [13,14] as well as decreased first phase insulin response (FPIR) in an intravenous glucose tolerance test (IvGTT) [15]. A risk score in first degree relatives based on metabolic markers from OGTT combined with age and BMI has been proposed to identify individuals who develop diabetes within the next two years [16]. An expanded definition of dysglycemia, beyond fasting and 120-minute plasma-glucose defining impaired fasting glucose and impaired glucose tolerance [17], was introduced with additional

inclusion of plasma-glucose ≥11.1 mmol/L at 30, 60 and/or 90 minutes during OGTT, indicating that these values may be added as predictive markers for type 1 diabetes [13].

Secondary prevention studies are complicated by the fact that the progression to clinical onset of diabetes may have reached, already at baseline, different levels of beta-cell function and dysglycemia. In our on-going Diabetes Prevention – Immune Tolerance (DIAPREV-IT) study, reduced glucose metabolism defined as low FPIR and/or high120-minute plasma-glucose on OGTT, was evident in 20/49 subjects enrolled [18]. We therefore hypothesized that baseline characteristics may predict the clinical onset of diabetes after two years of follow-up, regardless of randomization.

The aim of the present study was to analyse and compare the time predictive value of 1) HbA1c and metabolic variables derived from IvGTT and OGTT, 2) measures of insulin resistance, and 3) number, type and levels of islet autoantibodies, all measured at baseline screening in a prevention study of non-diabetic children with multiple islet autoantibodies.

Materials and Methods

Study population

In DiAPREV-IT (EuCT 2008-007484-16, NCT01122446), 50 non-diabetic children (27 boys, 23 girls) aged 4-17.9 years with GAD65A and at least one more islet autoantibody (IA-2A, ZnT8 RA, QA, WA or IAA) were included between April 2009 and January 2012 [18]. Both first-degree relatives (n=16) and children from the general population (n=34) were included. All children were, after baseline staging with both IvGTT and OGTT at two separate visits maximum 35 days apart, randomized for treatment with Alum-GAD65 (Diamyd®, Diamyd Medical, Sweden) (n=25) or placebo (n=25). The DiAPREV-IT study is still blinded and the children will be followed for five years.

OGTT (120 minutes, five time-point) was performed at baseline in all but one participant (one boy refused). After fasting overnight, Nutrical® (N.V. Nutricia Zoetermeer, Holland) was given orally in a dose of 1.75 g glucose/kg body weight. Venous plasma-glucose, serum-C-peptide and insulin were measured at 0, 30, 60, 90 and 120 minutes. Venous plasma-glucose <7.8 mmol/L at 120 minutes on OGTT was considered normal, while impaired glucose tolerance was defined as plasma-glucose ≥7.8 and <11.1 mmol/L. Plasma-glucose ≥11.1 mmol/L indicated diabetes [17].

IvGTT (90 minutes) was performed in all participants at baseline. After fasting overnight, glucose (500 mg/kg body weight) was injected within 3 minutes as a 30% solution. Venous plasma-glucose, serum-C-peptide and insulin were measured at -10, 0, 1, 3, 5, 7, 10, 30, 50, 70 and 90 minutes and glucose values were plotted semi-logarithmically against time. The disappearance rate of glucose was estimated on the near-straight line to calculate the K-value [19]. FPIR (the sum of serum-insulin at the one and three minutes time points after the infusion of glucose) ≤30 µU/mL were considered as a marker of reduced beta-cell function [19] .

HbA1c, serum-insulin and serum-C-peptide: The Department of Laboratory Medicine, Skåne University Hospital analysed HbA1c in the IFCC VARIANT ™ TURBO HemoglobinA1c Kit- 2.0 program (Bio-Rad Laboratories, Hercules, CA),and serum-insulin and C-peptide in an ElectroChemiLumiscenceImmunoassay (Cobas NPU02496 and NPU04149).

Autoantibodies: Autoantibodies to GAD65, IA-2 and the three amino acid variants at position 325 of the ZnT8 transporter were determined as described [18,20]. IAA was analysed as detailed elsewhere [18] with the following modifications: bovine serum albumin (1% w/v) was added the incubation buffer (TRIS buffer (pH 8.0) with 1% (v/v) Tween 20) to prevent non-specific binding.

Instead of arbitrary units, U/mL was calculated using a standard curve diluted in seven different concentrations (range 358 – 3 U/mL), plotted against cpm values on a Log2 scale.

HLA genotyping was performed with allele specific probes for HLA-DQ A1 and B1 alleles as described in detail [20].

BMI standard deviations (BMI SD): Height and weight was measured at both baseline visits. BMI SD was calculated according to the Swedish index reference values for all visits as described [21].

HOMA-index was calculated according to the following formula: fasting venous plasma-glucose (mmol/L) x fasting serum-insulin (µU/mL)/22.5.

Statistical methods: Statistical analyses were done with R, using the survival package. Cox proportional hazard analyses were done with the coxph function using the default settings and survival curves were compared doing the log-rank test with the survival difference function. Metabolic variables measured at baseline were used both as continuous and as dichotomized variables.

Results

A total of 50 children (27 boys) were included in the DiAPREV-IT study. One 11-year-old female was excluded from these analyses due to diabetes values on baseline OGTT. The median age at baseline of the remaining 49 children was 5.1 years (range 4.0-17.8) and 15 (31%) were first-degree relatives to type 1 diabetes patients. All but three had high to moderate risk HLA genotypes and 24/49 (49 %) had the high risk HLA-DQA1*05:01-B1*02:01/DQA1*03:01-DQB1*03:02 (DQ2/8). None of the children had DQB1*06:02. Baseline characteristics of the 49 children are described in Table 1. There were no significant differences between first-degree relatives and children from general population in autoantibody pattern or glucose metabolism at baseline screening. At the time for this analysis all children had been followed for a minimum of two years from baseline and 12/49 children (4 boys) had progressed to clinical type 1 diabetes. All were diagnosed according to ADA criteria [17], but at an early stage of disease during the intense follow-up in the study; 9/12 (75 %) had no symptoms and none of the children had diabetes ketoacidosis. Median time between inclusion and diagnosis for children who developed diabetes was 719 days (range 411-1105) (Table 1).

Time to diabetes and characteristics at baseline: No significant increase in risk was found with age at screening (HR 1.00 (95% CI 0.998-1.00), p=0.173), the child being first-degree (HR 1.17 (CI 0.351-3.89), p=0.800), or having the HLA high-risk genotype DQ2/8 (HR 1.68 (CI 0.379-5.29), p=0.379). BMI SD was not associated with diabetes risk (HR 0.922 (CI 0.527-1.61), p=0.776).

Time to diabetes and glucose metabolism at baseline: The only metabolic variables indicating risk for progression to type 1 diabetes were FPIR from IvGTT (p=0.002), also if adjusted for age (p=0.005), and plasma-glucose AUC from OGTT (p=0.012) (Table 2a). Only one of the children who developed diabetes had plasma-glucose AUC below median, and all but three children had FPIR below median (data not shown). Serum-insulin AUC from OGTT as risk indicator could not replace FPIR from IvGTT. The increased risk of OGTT plasma-glucose AUC indicates that the glucose levels during the test may be of relevance, as suggested in a previous study [13].

We next analysed the risk of diabetes with a maximal plasma-glucose ≥11.1 mmol/L at any sample during the baseline OGTT (at 30, 60 and/or 90 minutes) (Table 2b). Children with a value above this threshold had a significantly increased risk of progression to diabetes (HR 6.13 (CI 1.79-21.0), p=0.0039). This was also found when FPIR was categorized as above or below 30 µU/ml (HR 4.42 (CI 1.40-14.0), p=0.0112) but not with the OGTT 120 minute plasma-glucose ≥7.8 mmol/L (HR

0.87 (CI 0.19-4.05), p= 0.859)(Table 2b). Using the maximal OGTT plasma glucose level ≥11.1 mmol/L as a variable, actually resulted in the highest HR and the lowest p-value of the variables tested. In combining low FPIR with maximal plasma-glucose ≥11.1 mmol/L during OGTT we captured 10/12 children developing diabetes. By also including 120-minute plasma-glucose ≥7.8 mmol/L we found 11/12 of the children developing diabetes. However, this also led to a larger increase in the total number of children (25/49) defined as having impaired glucose metabolism at baseline, reflected by the increase in p-value in the Cox model when including the OGTT 120-minute value into the definition for impaired glucose metabolism.

Kaplan-Meier curves of the time to diabetes using the categorized baseline variables from Table 2, illustrated the increased risk with low FPIR (≤30 μU/mL) from IvGTT (p=0.00562) and maximal plasma-glucose ≥11.1 mmol/L during OGTT (p=0.000994), while no increased risk was seen for a 120-minute plasma-glucose on OGTT ≥7.8 mmol/L (p=0.859) (Figure 1). When combining FPIR with maximal plasma-glucose, the p-value of a log-rank test was slightly lower (p=0.000529). Inclusion of 120-minute plasma-glucose did not cause any major change of the survival curves, although one more child developing diabetes was captured (Figure 1). Thus, including the maximal plasma-glucose during OGTT was important for estimating risk in this young cohort of children.

Time to diabetes and autoantibodies: Since the autoantibody-levels varied between the first and second baseline visit, we used the average value of the two visits in these analyses. The child had to be positive for GAD65A and at least one more islet autoantibody at the first baseline visit to be eligible for inclusion. One child had lost GAD65A at the second baseline visit with an average level below the cut-off for positivity, and was therefore negative in this analysis (Table 1).

The number of autoantibodies was not associated with time to diagnosis of type 1 diabetes (data not shown). Positivity and levels of autoantibodies to GAD65A, IAA, ZnT8RA, ZnT8WA and ZnT8QA were equally distributed between children who did not and did develop diabetes, while all but one child developing diabetes were positive to IA-2A and above median in IA-2A level (Figure 2a). IA-2A levels above median increased the risk for progression to type 1 diabetes (Cox regression: HR 4.98 (CI 1.09-22.8), p=0.039). Survival curves showed p=0.0218 in the log rank test (Fig 2b).

Levels of autoantibodies and glucose metabolism at baseline: The predictive information of dysglycemic indicators raised the question whether these parameters were associated with islet autoantibodies. It was found that levels of IA-2A above median were associated with maximal plasma-

glucose ≥11.1 mmol/L during OGTT (p=0.008), but not with FPIR or 120-minute plasma-glucose. Additionally, levels of IAA above median were found to be associated to low FPIR (p=0.02). No other association between levels of autoantibodies and glucose metabolism at baseline was found.

Discussion

The main findings in this study in non-diabetic young children with two or more islet autoantibodies, recruited mainly from general population, was that maximal plasma-glucose values ≥11.1 mmol/L 30/ 60/90 minutes during OGTT was the most predictive test for the diagnosis of type 1 diabetes during follow-up. The FPIR from IvGTT was also prognostic and a combination of these two variables may be an optimal indicator. The conventional definition of IGT, with a 120–minute plasma-glucose ≥7.8 mmol/L on OGTT, did not predict the diagnosis in this cohort of young children, indicating the importance of including the 30, 60 and 90 minutes plasma-glucose values during OGTT for adequate prediction. A major finding was also that high-level IA-2A at baseline was associated with both dysglycemia during OGTT and an increased risk of progression to type 1 diabetes.

Although our study only included 50 children, the dual testing with IvGTT and OGTT made it possible to demonstrate a significantly increased risk of diabetes with decreased FPIR, maximal plasma-glucose during OGTT and high IA-2A levels. We therefore confirm a similar observation in an older cohort with family history of diabetes [13]. It is interesting to note that although these children had signs of impaired beta-cell function at baseline, it took more than a year until the first child was diagnosed according to the ADA criteria. This indicates that metabolic changes can be seen long before the diagnosis in very young children.

The present study included primarily children from the general population with no immediate family history of diabetes, since the majority of patients developing type 1 diabetes have no close relative with the disease [18]. In contrast to previous findings where first degree relatives have an increased risk of developing autoantibody positivity and type 1 diabetes at an early age [22], no difference was seen in risk of progression to diabetes between children with a first degree relative with type 1 diabetes (n=15) compared to children from the general population (n=34). Since all but one of the children developing diabetes had at least one sign of impaired glucose metabolism, this may imply that when the autoimmune process has progressed to that stage, the genetic risk or family history of type 1 diabetes had a lesser influence. This finding is coherent with a recent study, where progression to type 1 diabetes in children followed with multiple islet autoantibodies was investigated. Rate of progression was similar between the different cohorts of children, composed of either primarily first-degree relatives or primarily children at genetic risk from general population [1].

The strengths of our study include the possibility to compare measures from IvGTT and OGTT, performed at two baseline visits with a maximum of 35 days apart, along with six different islet autoantibodies at both occasions. In this way it was possible to establish the following measures of diabetes risk: maximal plasma-glucose ≥11.1 mmol/L 30/60/90 minutes during OGTT > FPIR > high level IA-2A > plasma-glucose AUC OGTT.

The fact that we were able to document major differences between the children already at baseline [18] prompted the present study. Previous studies have analysed predictive values of IvGTT [15,23], OGTT [13] and autoantibody levels [2,11,14,13] separately, while one study used prediction models with a number of different variables [16]. In our study, 20/49 (41%) children were initially found to have impaired glucose metabolism at baseline based on FPIR and 120-minute plasma-glucose [18]. However, when adding the maximal plasma-glucose 30/60/90 minutes during OGTT, 25/49 (51%) had some signs of impaired glucose metabolism at baseline, including the 11/12 children developing diabetes up to date. An impaired glucose tolerance alone, defined as 120-minute plasma-glucose ≥7.8 mmol/L at baseline OGTT, did not significantly increase the risk, while a low FPIR and maximal plasma-glucose ≥11.1 mmol/L during OGTT did, indicating that IvGTT and fully analysed OGTT is important for the accurate risk-score of autoantibody positive young children.. Our findings have to be related to the relatively small number of participants in the study and that the children are participating in a, still blinded, prevention study.

High titres [24] as well as increasing titres [25] of IA-2 have previously been found to predict clinical onset of type 1 diabetes. We could confirm this finding in our young population of GAD65A positive children. As all the children, but one, who developed diabetes had IA-2A above the median value, our study implicates that also a moderate increase in IA-2A is an important risk factor, further stressed by the finding that IA-2A levels above median were associated with maximal plasma-glucose ≥11.1 mmol/L during OGTT. Once the child had developed dysglycemia, high-level IA-2A did not act as a strong additional risk factor for progression to type 1 diabetes. Hence, high-level IA-2A levels may not be an independent risk factor for progression to type 1 diabetes as previously reported [26,24] but rather be related to and precede dysglycemia observed prior to the clinical onset of type 1 diabetes.

We previously reported that positivity for ZnT8QA were associated with decreased glucose metabolism at baseline inclusion in the study [18] and we could now confirm that positivity, but not levels, for both ZnT8WA and ZnT8QA was associated with the extended definition of impaired

glucose metabolism (p=0.022 and 0.046 respectively). However, none of the ZnT8A was associated with progression to type 1 diabetes.

No increased risk of type 1 diabetes was seen with increasing number of autoantibodies or type of autoantibodies. This finding is contradicting previous findings that numbers of autoantibodies are important for the risk of type 1 diabetes [11]. However, since the autoantibodies have been shown to have a time-dependent pattern of appearance and disappearance [2,25], previously positive autoantibodies may have been lost during the autoimmune process and other autoantibodies becoming positive prior to the baseline visit. It is also important to note that the children participating in this study all had at least two autoantibodies at baseline due to the inclusion criteria, which may affect the results.

In contrast to previous studies [16,23,15] we did not find any association between BMI SD or HOMA-Index, as a measure of insulin resistance and previously included in risk models [15,23], and progression to diabetes. This may be explained by our young cohort of children, (median age 5.1 years), which may be less affected by risk factors such as insulin resistance and BMI SD. Therefore we suggest that insulin resistance is of minor importance in our cohort of young children, further demonstrated by the importance of FPIR in contrast to HbA1c and 120-minute glucose value on OGTT for the risk of disease.

We conclude that maximal plasma-glucose values ≥11.1 mmol/L at 30/60/90 minutes during OGTT as well as low FPIR from IvGTT were the most important predictive test for progression to diabetes in our study of young children with multiple islet autoantibodies. High IA-2A levels were related to dysglycemia and may therefore not be an independent risk factor despite it was more significant for prediction than the number of islet autoantibodies.

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Appendix:

Members of the DiAPREV-IT study group are: PI: Helena Elding Larsson (Malmö), Co-PI: Åke Lernmark (Malmö), Study nurses: Caroline Nilsson, Gertie Hansson.

Lab personnel: Jeanette Arvastsson, Rasmus Bennet, Charlotte Brundin, Ida Jönsson, Zeliha Mestan, Anita Ramelius, and Ingrid Wigheden.

Physicians: Cecilia Andersson (Malmö), Susanne Bach Meineche (Malmö), Annelie Carlsson (Lund), Elisabeth Cederwall (Ängelholm), Corrado Cilio (Malmö), Sten Ivarsson (Malmö), Berglind Jonsdottir (Malmö), Björn Jönsson (Ystad), Karin Larsson (Kristianstad), Bengt Lindberg (Malmö), Markus Lundgren (Kristianstad), Jan Neiderud (Helsingborg), Ann Olsson (Trollhättan), Lars Åke Persson (Uppsala), Eva Örtqvist (Stockholm).

Contribution statement:

Helena Elding Larsson designed the study, collected and interpreted data and wrote and revised the manuscript, Christer Larsson analysed and interpreted data and revised the manuscript, Åke Lernmark designed the study and carefully revised the manuscript.

Conflict of interests: Helena Elding Larsson, Christer Larsson and Åke Lernmark declare that they have no conflict of interests.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1976, as revised in 2008. Informed Consent was obtained from all participant or their legal representatives for being included in the study.

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Figure 1. Time to diabetes onset from baseline in children related to metabolic values from IvGTT and OGTT; a) First phase insulin response (FPIR) ≤30 µU/mL from IvGTT was associated with higher risk of diabetes diagnosis (p=0.00562), b) maximal plasma-glucose ≥11.1 mmol/L at 30, 60 and/or 90 min during OGTT increased the risk of diabetes (p=0.000994), c) No significant increase in risk to be diagnosed with diabetes was seen with 120-minute plasma-glucose ≥7.8 mmol/L on OGTT (p=0.859), d) Combined analysis of FPIR and maximal plasma-glucose ≥11.1 mmol/L at 30, 60 or 90 minutes during OGTT predicted development of diabetes in 10/12 children, e) A combined analysis of FPIR, maximal plasma-glucose ≥11.1 mmol/L at 30, 60, 90 and 120-minute plasma-glucose ≥7.8 mmol/L during OGTT predicted 11/12 children developing diabetes.

Figure 1

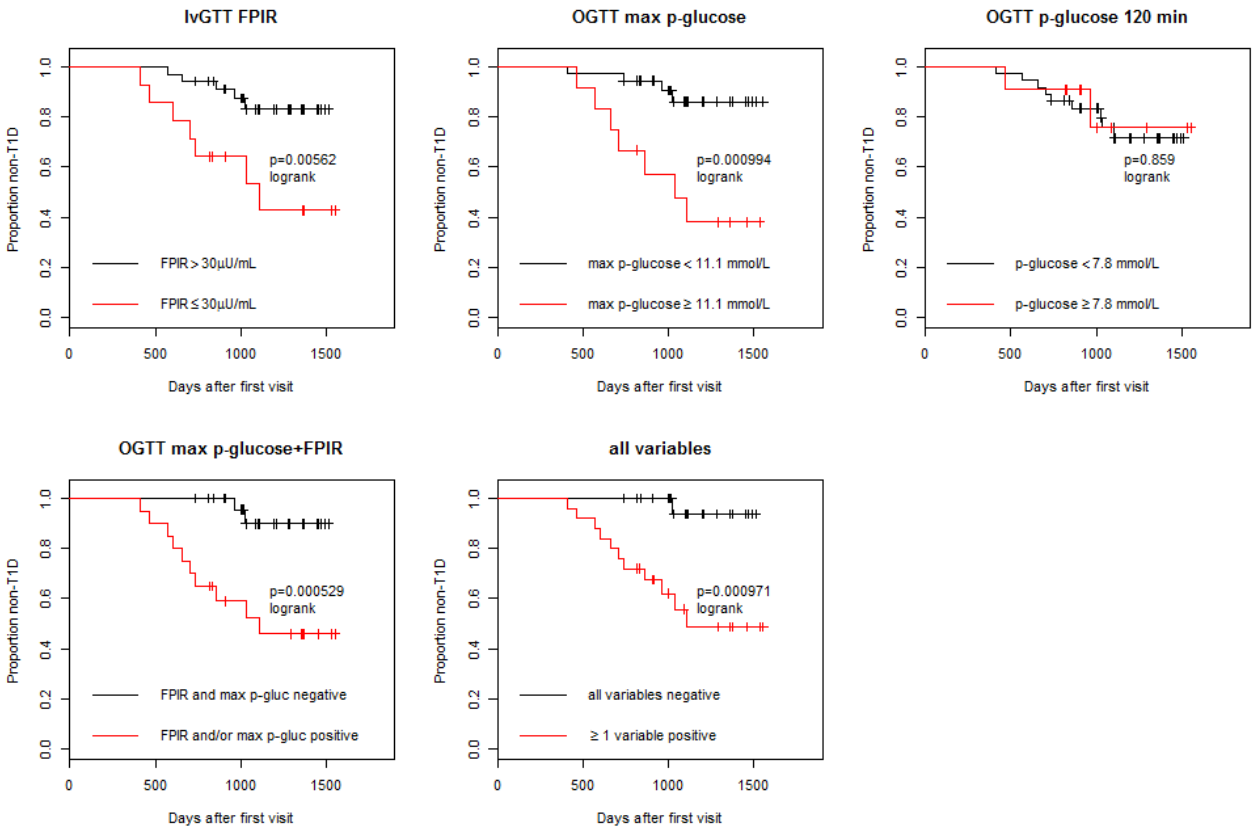

Figure 2a) Positivity and titres of autoantibodies to GAD65A, IAA, IA-2A, ZnT8RA, ZnT8WA and ZnT8QA distributed between children who did not (green) and did (red) develop diabetes. All but one child developing diabetes were positive to IA-2A and above median in IA-2A titre. The dotted line represents the cut-off level for positive autoantibodies, the dashed line indicates the median level in the cohort. Figure 2b) Kaplan Meyer of IA-2A levels above and below median and risk of developing type 1 diabetes.

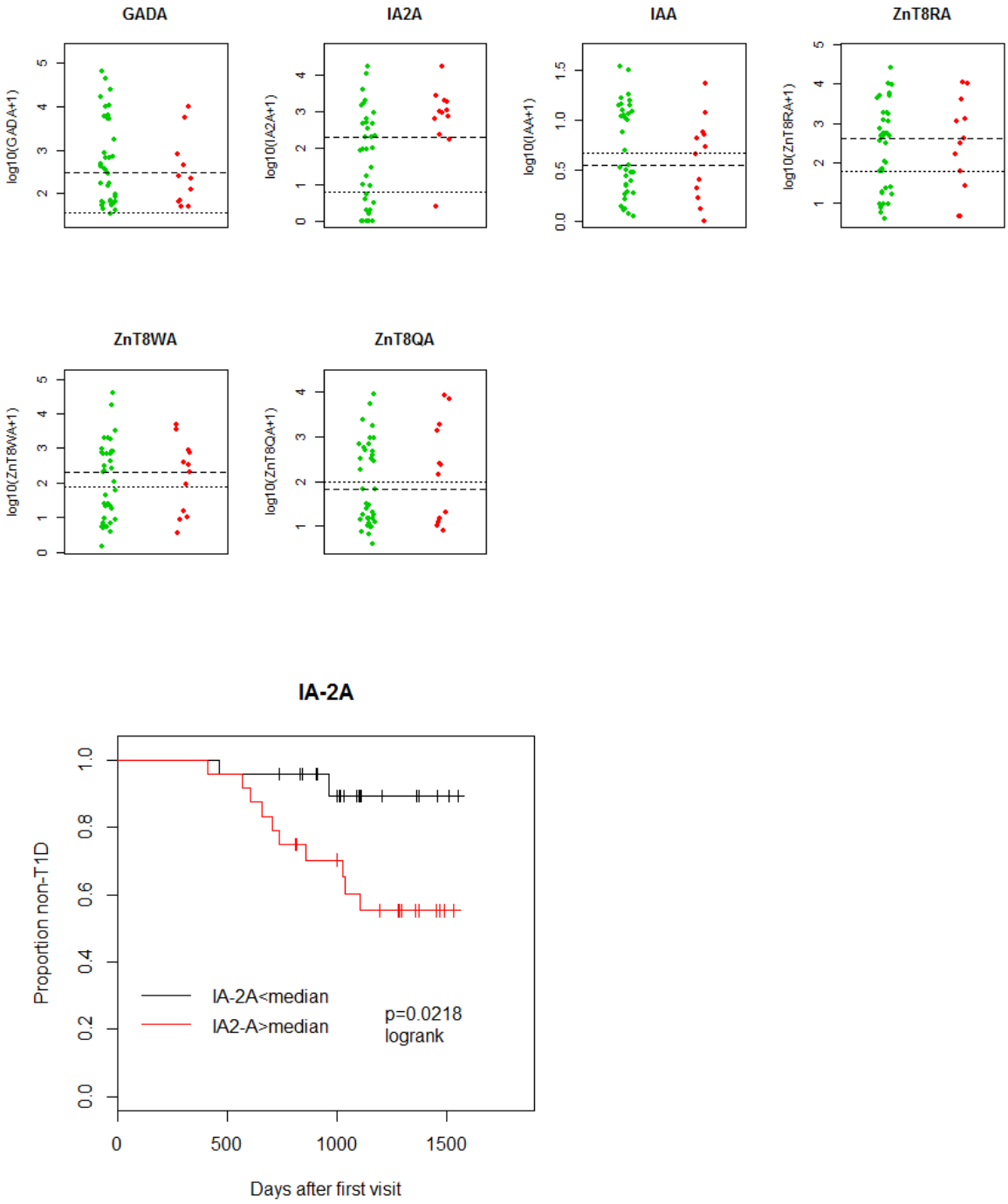

Table 1. Baseline data of the 49 non-diabetic children recruited to the clinical trial Diabetes Prevention-Immune Tolerance (DiAPREV-IT) and relation to diagnosis of diabetes.

	All Subjects	Remained healthy	Developed diabetes
Number	49	37	12
Median age in years (%)	5.1 (4.0-17.8)	5.5 (4.0-17.8)	4.9 (4.0-7.2)
Gender: male n (%)	27 (55%)	23 (62%)	4 (33%)
First-degree relatives n (%)	15 (31%)	11 (30%)	4 (33%)
BMI SDS median (range)	0.26 (-1.65-1.96)	0.26 (-1.65-1.96)	0.21 (-1.47-1.84)
HLA-DQ n (%)			
2/8	24 (49%)	17 (46%)	7 (58%)
8/8, 8/X	20 (41%)	16 (43%)	4 (33%)
2/2, 2/Y	3 (6%)	2 (5%)	1 (8%)
Z/Z	2 (4%)	2 (5%)	0 (0%)
Islet autoantibodies n (%) (average visit 0 and 1)			
GAD65A*	48 (98%)	36 (97%)	12 (100%)
IA-2A	35 (71%)	24 (64%)	11 (92%)
IAA	23 (47%)	17 (45%)	6 (50%)
ZnT8WA	32 (65%)	24 (65%)	8 (67%)
ZnT8RA	27 (55%)	19 (51%)	8 (67%)
ZnT8QA	24 (49%)	17 (46%)	7 (58%)
Time (days of follow-up)		1110 (736-1553)	719 (411-1105)

X is not 2, Y is not 8, Z is neither 2 nor 8
*Positive GAD65A at screening was inclusion criteria for participation. One child was positive at screening while negative at visit 1, and therefore in this analysis deemed negative.

Table 2: Baseline metabolic variables from 49 non-diabetic children with GADA and at least one more islet autoantibody were analysed with cox proportional hazard regression, after standardization of the variables, with type 1 diabetes as endpoint.

2a) Indicated metabolic variables measured at the baseline visit used as continuous variabes in Cox proportional hazard analysis for risk of type 1 diabetes. 2b) Indicated IvGTT and OGTT variables were dichotomized as shown and used in cox proportional hazard analysis for risk of type 1 diabetes.

Table 2a

	HR	95% CI	p-value
HbA1c	1.66	0.88-3.11	0.117
Fasting plasma-glucose	1.13	0.66-1.94	0.655
Fasting serum-C-peptide	0.82	0.42-1.60	0.555
Fasting serum-insulin	1.30	0.77-2.19	0.322
HOMA-IR	1.10	0.65-1.85	0.729
IvGTT:			
FPIR	**0.08**	**0.01-0.40**	**0.002**
K-value	0.79	0.42-1.48	0.466
Plasma-glucose AUC	1.72	0.96-3.10	0.069
Serum-C-peptide AUC	0.56	0.24-1.31	0.181
Serum-insulin AUC	0.44	0.14-1.37	0.157
OGTT:			
120-minute plasma-glucose	1.44	0.82-2.53	0.204
Plasma-glucose AUC	**1.98**	**1.16-3.39**	**0.012**
Serum-C-peptide AUC	0.63	0.28-1.46	0.283
Serum-insulin AUC	0.74	0.34-1.59	0.440

Table 2b (n=48/49, diabetes cases n=11/12*)

	HR	95% CI	p-value
FPIR ≤30 µU/mL	**4.42**	**1.40-14.00**	**0.0112**
120-minute plasma-glucose ≥7.8 mmol/L on OGTT	0.87	0.19-4.05	0.8590
Maximal plasma-glucose ≥11.1 mmol/L at 30, 60, 90 min during OGTT	**6.13**	**1.79-21.00**	**0.0039**
FPIR ≤30 µU/mL and/or maximal plasma-glucose ≥11.1 mmol/L 30, 60,90 min during OGTT	**9.17**	**2.00-42.00**	**0.0043**
At least one of above variables	**13.90**	**1.79-108.00**	**0.0118**

*One child excluded in this analysis, due to refusal to perform OGTT.