HbA1c Predicts Time to Diagnosis of Type 1 Diabetes in Children at Risk

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Prediction of type 1 diabetes is based on the detection of multiple islet autoantibodies in subjects at increased genetic risk. Prediction of the timing of diagnosis is challenging, however. We assessed the utility of HbA1c in predicting the clinical disease in genetically predisposed children with multiple autoantibodies. Cord blood samples from 168,055 newborn infants were screened for class II HLA genotypes in Finland, and 14,876 children with increased genetic risk for type 1 diabetes were invited to regular follow-up including screening for diabetes-associated autoantibodies. When two or more autoantibodies were detected HbA1c was analyzed at each visit. During follow-up 466 children developed multiple (≥2) autoantibodies, and 201 (43%) of these were diagnosed with type 1 diabetes (progressors), while 265 remained disease-free (non-progressors) by December 2011. A 10% increase in HbA1c levels in samples taken 3-12 months apart predicted the diagnosis of clinical disease (HR 5.7, 95% CI 4.1-7.9), after a median time of 1.1 years (IQR 0.6-3.1) from the observed rise of HbA1c. If HbA1c was ≥5.9% (41 mmol/mol) in two consecutive samples, the median time to diagnosis was 0.9 years (IQR 0.3-1.5; HR 11.9, 95% CI 8.8-16.0). In conclusion, HbA1c is a useful biochemical marker when predicting time to diagnosis of type 1 diabetes in children with multiple autoantibodies.
The development of type 1 diabetes is characterized by immune-mediated destruction of the pancreatic β-cells which eventually stop to produce insulin. The incidence of type 1 diabetes has been growing worldwide, with the highest incidence rate observed in Finland where the rate has more than doubled from 1980 to 2005 in children under the age of 15 years (1-3).

More than 50 genes conferring susceptibility to human type 1 diabetes have been identified (4). The major risk genes are located in the class II HLA region on chromosome 6p21 (5). Environmental risk factors are believed to interact with susceptibility genes and thereby contribute to the disease process (6, 7). Currently, type 1 diabetes is predicted by analyzing islet autoantibodies of various specificities in an individual with increased genetic disease susceptibility. The presence of multiple (≥2) autoantibodies provides a cumulative disease risk of 50-60% over the next 5 years in children with risk-conferring HLA class II genotypes (8) and during 15-year follow-up the risk of developing diabetes is very high, 84%, in such children (9).

Although oral glucose tolerance tests (OGTTs) have been shown to provide a relatively good accuracy in the prediction of type 1 diabetes (10, 11), more practical markers of glucose metabolism are needed. Glycated hemoglobin A1c (HbA1c) could be superior to OGTT as a predictive measure, since it is widely used in clinical practice as an indicator of metabolic control in patients with diabetes and is generally not affected by short-term variations in food intake and physical activity (12, 13). World Health Organization (WHO) and American Diabetes Association (ADA) have adapted the International Expert Committee’s recommendation that a HbA1c value ≥6.5% (48mmol/mol) is diagnostic for type 2 diabetes (14) but no threshold value for the diagnosis of type 1 diabetes has been determined (15). As a laboratory test HbA1c is cost effective and easy to obtain, and it could be useful also in the prediction of type 1 diabetes (16). Although positivity for multiple islet autoantibodies has been shown to be relatively sensitive in prediction of type 1 diabetes, there is a lack of a reliable method to predict the onset of type 1 diabetes. In this study the goal was to
monitor HbA1c from the start of positivity for multiple (≥2) autoantibodies and to assess its potential as a predictive marker for type 1 diabetes.

**RESEARCH DESIGN AND METHODS**

**Study design**

The Type 1 Diabetes Prediction and Prevention study (DIPP) is a Finnish population-based study in which children with HLA-conferred susceptibility to type 1 diabetes are observed prospectively from birth (17, 18). Recruitment of newborn infants for the DIPP study started in November 1994, and still continues in three university hospitals (Oulu, Tampere and Turku) in Finland. Screening for genetic risk is performed from cord blood. Families with an infant carrying HLA genotypes associated with type 1 diabetes are invited to prospective follow-up at 3 to 12-month intervals until the age of 15 years or until clinical disease is diagnosed. Islet autoantibodies are analyzed at each visit and monitoring of HbA1c is initiated in children who test positive for at least two different autoantibodies in the same sample, and positivity is confirmed in the subsequent sample. The diagnosis of type 1 diabetes is based on typical symptoms and random high plasma glucose levels, or in an asymptomatic patient diagnostic plasma glucose values in two separate oral glucose tolerance tests as WHO recommends (19). The study population for the current analysis comprises 466 DIPP children who tested positive for multiple autoantibodies in a minimum of two consecutive samples and had at least one HbA1c sample taken between November 1994 and December 2011. The study has been approved by the Ethics Committees of the participating university hospital districts. All families participating in the study have provided written informed consent.

**Immunological screening**

DIPP participants who seroconverted to positivity for any of the diabetes-associated autoantibodies; islet cell antibodies, insulin autoantibodies and GAD antibodies or the insulinoma-2 associated antigen (IA-2) were scheduled for follow-up visits at 3-month intervals. The age at seroconversion
was defined as the age at which at least one of the diabetes-associated autoantibodies was detected for the first time. The age at multiple autoantibody positivity was defined as the time point, when at least two autoantibodies were detected in the same sample.

Autoantibodies were analyzed as described previously (8). In the Diabetes Autoantibody Standardization (DASP) workshop in 2005 the following sensitivities and specificities were reported: insulin autoantibodies 58% and 96%, GAD antibodies 82% and 96% and IA-2 antibodies 72% and 100%.

**Genetic screening**

HLA-conferred susceptibility to type 1 diabetes was analyzed centrally using cord blood as described earlier (20). According to various HLA DRB1–DQA1–DQB1 haplotype combinations genotypes conferring high, moderate and low risk for the disease were determined. High risk was defined as heterozygosity for the two risk-associated haplotypes DRB1*04:01/2/4/5/8-DQA1*03-DQB1*03:02/4 and [DRB1*03]-DQA1*05-DQB1*02. Moderate risk was defined as homozygosity for any of the two risk haplotypes or DRB1*04:01/2/4/5/8-DQA1*03-DQB1*03:02/4 combined with a neutral haplotype, or the [DRB1*03]-DQA1*05-DQB1*02/[DRB1*09]-DQA1*03-DQB1*03 genotype. Low risk was conferred by other genotypes as previously defined (21).

**HbA1c assays and measurements**

All HbA1c values were measured from venous blood samples in the clinical laboratories of the three university hospitals. Methods for HbA1c analyses varied slightly between the centers.

In the Oulu University Hospital an immunoassay-based method was applied throughout the study. Until May 6, 2004 Cobas Integra 700 (F.Hoffman-La Roche Ltd, Basel, Switzerland) was used with the reagents HbA1c 2054302 (F.Hoffman-La Roche Ltd). From May 7, 2004 until April 20, 2009
Advia 2400 (Siemens, Munich, Germany) was used with the reagents Bayer B01-4797-01 (Bayer Healthcare, Leverkusen, Germany). From April 20, 2009 onwards Advia 1800 (Siemens) has been used with the reagents HbA1c 06854744 (Siemens).

In the Tampere University Hospital Fast Protein Liquid Chromatography (FPLC) was used to measure HbA1c until June 9, 1999 (Pharmacia, Uppsala, Sweden, with the reagents Pharmacia Mono S for HbA1c, 17-1040-01. From June 10, 1999 until November 14, 2007 an immunoassay-based method was used (Cobas Integra with the reagents Hemoglobin A1C, F.Hoffman-La Roche Ltd). From November 15, 2007 onwards Cobas Integra has been used with the reagents Tina-quant Hemoglobin A1c Gen 2.

In the Turku University Hospital the FPLC method was used to measure HbA1c until August 5, 1996 (Pharmacia). Thereafter High Performance Liquid Chromatography (HPLC) has been used. From August 6, 1996 until July 13, 2001 the Variant Classic instrumentation (BIO-RAD, CA, USA) was used with the reagents VARIANT HbA1c Reorder Pack no 270-0003 (BIO-RAD). From July 14, 2001 until November 20, 2007 the Variant II instrumentation, (BIO-RAD) was used with the reagents VARIANT II HbA1c Reorder Pack no 270-2101 (BIO-RAD). From November 21, 2007 until October 13, 2008 the Variant II instrumentation (BIO-RAD) was still used but with the reagents VARIANT II HbA1c Reorder Pack no 270-2101 NU (BIO-RAD). From October 14, 2008 until March 2, 2010 the Variant II Turbo instrumentation (BIO-RAD) was used with the reagents VARIANT II Turbo HbA1c Reorder Pack no 270-2415 (BIO-RAD) which were changed to VARIANT II Turbo HbA1c Kit – 2.0, REF 270-2455EX (BIO-RAD) starting from March 3, 2010.

The mean of all HbA1c measured in the Oulu University Hospital was 5.57% (SD 0.78), in Tampere 5.56% (SD 0.46) and in Turku 5.57% (SD 0.51). The covariate adjustment was used in the linear mixed model analysis to take into account the possible confounding effect of variable assay
levels in the three hospitals.

**Statistical analyses**

We established *a priori* three time-dependent decision rules for HbA1c. First, children who had at least two HbA1c measurements within 3-12 months were classified into two groups according to whether they experienced an increase of at least 10% in their HbA1c values or not (decision rule 1). Second, children who had at least two HbA1c measurements within 3-12 months and a third HbA1c measurement during the next 6 months were classified into two groups according to whether they had a 10% increase in HbA1c values within 3-12 months and any additional increase during the next 6 months or not (decision rule 2). Thirdly, children were divided into two groups according to whether they had two consecutive HbA1c values ≥5.9% (41 mmol/mol) or not (decision rule 3). The use of an increase of 10% as a decision rule was based on the data from the three laboratories involved. The inter-assay coefficient of variation (CV) was 3.3% in Oulu, 2.0% in Tampere and 2.7% in Turku, and the reported uncertainty of measurements was 10%, 5.8% and 5.9%, respectively. The calculation of the uncertainty was based on current recommendations (22). The highest reported percent of uncertainty was selected for the analysis. The cut off HbA1c value of ≥5.9% (41 mmol/mol) has been suggested in previous studies on prediction of diabetes (23-25).

Two sequential measurements were required to minimize the biological and analytical variation. A time window of 3-12 months was used in the analyses according to the DIPP protocol in which follow-up visits occur every 3 months.

Cox regression with these time-dependent covariates was used to evaluate the association between HbA1c and the risk of diabetes. The entry time to the analysis was the date when at least two islet autoantibodies were positive. All subjects were considered to be unexposed until the time-dependent covariate fulfilled the decision rule, and thereafter the status of the subject was exposed. Consequently, if the subject had only one (decision rule 1 and decision rule 3) or two (decision rule
2) HbA1c measurements he/she belonged to the unexposed group. Samples taken on the day of
diagnosis were excluded from the analyses considering the predictive use of HbA1c (decision
rules), but not from the linear mixed model (LMM) analysis. The total follow-up time was
partitioned into intervals according to cut points of the decision rule. Univariate Cox regression
analysis was performed to identify risk factors of diabetes and survival curve estimates were
calculated to assess the timing and probability of diabetes after fulfilling a decision rule. Sensitivity
and specificity for the three decision rules were also calculated. Multivariate Cox regression with
the backward stepwise model was used to identify a set of predictors most effective in disease
prediction. In the multivariate analysis the explanatory variable candidates were a 10% increase in
HbA1c values within 3-12 months, a 10% increase in HbA1c values within 3-12 months and any
additional increase during the next 6 months, two consecutive HbA1c values ≥5.9% (41 mmol/mol),
age at multiple autoantibodies, type 1 diabetes in a first-degree relative (FDR), HLA risk and
gender.

LMM with random intercept and first-order autoregressive covariance structure for repeated
measurements was used to analyze HbA1c levels over time between the progressors and non-
progressors. The random intercepts and repeated measurements were nested within subjects and
subjects were nested within hospital. The group-by-time interaction was included in the model to
test differences between group means at each time point. Gender, age at sampling, age at
seroconversion, age at multiple autoantibody positivity, FDR and HLA risk were included in LMM
model as fixed variables.

Differences in proportions were tested using the Standardized normal deviate (SND) test.

All analyses were performed using IBM SPSS Statistic 20.0.0 for Windows, Stata/IC 11.2 for
Windows and StatsDirect statistical software 2.7.9. The figures were drawn using OriginPro 8.6.0
RESULTS

Between November 1994 and December 2011 a total of 168,055 newborn infants were screened for HLA-confferred susceptibility to type 1 diabetes. Altogether 14,876 children with increased genetic risk were enrolled for regular follow-up in the DIPP study. During follow-up 466 children developed multiple positive autoantibodies, and 201 (43%) of these progressed to type 1 diabetes (progressors) whereas 265 remained disease-free until the end of December 2011 (non-progressors). In the progressor group 136 children (68%) developed at least two positive autoantibodies in their first positive sample, whereas in the non-progressor group the number was 110 (42%), \( P<0.001 \).

The basic characteristics of the children are presented in Table 1.

Altogether 4270 HbA1c samples were analyzed during the prediabetic period, 1613 from the progressors and 2657 from the non-progressors. An average of 8.0 (95% CI 7.0-9.0, range 1-37) measurements per child were obtained in the group of progressors and 10.0 (95% CI 8.9-11.1, range 1-44) in the group of non-progressors.

When retrospectively comparing HbA1c levels between the progressors and non-progressors we observed that HbA1c started to be consistently higher in the progressors 2.0 years before the diagnosis (Fig. 1). More specifically, during the period 1.8-2.0 years before diagnosis the adjusted mean of HbA1c was 5.5% (37 mmol/mol), 95% CI 5.4-5.7 (36-39), among the progressors compared to 5.4% (36 mmol/mol), 95% CI 5.2-5.4 (33-36), in the non-progressors \( P=0.025 \).

During the following year a small but significant difference was consistently observed between the two groups. Thereafter the adjusted mean of HbA1c in the progressors started to increase steeper: 5.9% (41 mmol/mol) during the period 0.4-0.6 years before diagnosis, 6.1% (43 mmol/mol) during 0.2-0.4 years, and 6.8% (51 mmol/mol) during 0.01-0.2 years before diagnosis. At diagnosis the
adjusted mean of HbA1c in the progressors was 7.6% (60 mmol/mol), 95% CI 7.5-7.7 (58-61), compared to that of 5.5% (37 mmol/mol), 95% CI 5.4-5.6 (36-38), among the non-progressors in their last measurement (P<0.001). The range of crude HbA1c at diagnosis was 4.9-12.9% (30-117 mmol/mol) in the progressors and 4.4-6.5% (25-48 mmol/mol) in the non-progressors at the end of follow-up.

A 10% increase in the HbA1c values taken 3-12 months apart increased the disease risk almost six-fold (HR 5.7; 95% CI 4.1-7.9, Table 2.), and half of these children developed clinical disease within 1.1 years (IQR 0.6-3.1) (Fig. 2B) according to the univariate Cox regression analysis. When including any additional rise in HbA1c in a third measurement during the subsequent 6 months the HR was 5.1 (95% CI 3.6-7.2), and 50% of the children developed overt disease during the next 0.7 years (IQR 0.7-1.5) (Table 2., Fig. 2C). If a child was found to have two consecutive HbA1c ≥5.9% (41 mmol/mol) the risk of diabetes was almost 12-fold compared to the remaining children (HR 11.9; 95% CI 8.8-16.0), and the median time to type 1 diabetes was 0.9 years (IQR 0.3-1.5) (Table 2, Fig. 2D). The most effective set of predictive variables in multivariate Cox regression analysis are shown in Table 3.

Sensitivities and specificities were calculated for the suggested decision rules. A 10% increase in HbA1c during 3-12 months provided sensitivity of 57% (95% CI 50 to 64) and specificity of 66% (95% CI 60 to 72). When including an additional rise during the next 6 months sensitivity of the test was 22% (95% CI 17 to 29) and specificity 91% (95% CI 87 to 94). Two consecutive values of HbA1c ≥5.9% (41 mmol/mol) presented sensitivity of 42% (95% CI 35 to 49) and specificity of 86% (95% CI 82 to 90).

When multiple positive autoantibodies were detected an increase of 10% in HbA1c values appeared after a mean time of 2.5 years (SD 2.0). For an additional rise the time was 2.8 years (SD 2.1) and
with two HbA1c ≥5.9% (41 mmol/mol) the mean time was 3.4 years (SD 2.4).

HbA1c ≥6.5% (48 mmol/mol) was detected in 61% (122/201) of the progressors and in only 2% (5/265) of the non-progressors. However, 83% (101/122) of the HbA1c values ≥6.5% (48 mmol/mol) in the group of progressors were observed at the time of diagnosis.

**DISCUSSION**

The prediction of type 1 diabetes has so far been based mainly on the presence of islet autoantibodies in subjects at increased genetic risk (8, 26, 27). Young age at seroconversion has also a clear impact on the risk of progression to clinical disease (17). In addition to autoantibodies consecutive oral glucose tolerance tests have been of interest in the prediction of type 1 diabetes giving at best almost 90% accuracy during a 2-year follow-up among the relatives of patients affected by type 1 diabetes (10, 11, 28-31). When evaluating the potential of HbA1c as an additional marker in the prediction of type 1 diabetes we looked for new and practical ways to predict the timing of the diagnosis. Three new criteria were established, a 10% rise in HbA1c values taken 3-12 month apart, an additional rise during the subsequent 6 months, and two consecutive values ≥5.9% (41 mmol/mol) that could be used to predict the clinical disease in a child with multiple autoantibodies. These results are important since families with a child positive for multiple islet autoantibodies are concerned about the time remaining to clinical disease. They are well aware of the high disease risk and need expert counseling. Stable HbA1c in consecutive measurements suggest that the child is not going to present with overt diabetes shortly. In contrast an increase in HbA1c gives a warning of incipient disease which may help in early diagnosis and thereby reduce the risk of acute complications, such as diabetic ketoacidosis (32).

Age at multiple (≥2) autoantibody positivity, time from seroconversion to multiple autoantibodies, presence of affected first-degree relatives and a high risk HLA class II genotype have previously
been identified as risk factors for type 1 diabetes (8, 16, 17, 33). In our analyses age at multiple autoantibody positivity was a significant predictive factor both in uni- and multivariate analyses. Children with an affected FDR had more than a two-fold disease risk demonstrating that genetic factors do have an important effect also on progression from β-cell autoimmunity to clinical disease. The HLA class II genotype appeared to play a role in disease progression although with a lower hazard ratio than the HbA1c variables.

Our prediabetic cohort of 201 Finnish children with multiple autoantibodies represents the largest series of young children who have participated in a long-term intensive follow-up from birth to diagnosis of type 1 diabetes. Only one study on the evolution of HbA1c levels during prediabetes has been published earlier (16). Although that study included a relatively small number of 28 islet autoantibody positive children who progressed to clinical diabetes, the results were similar to our study showing that HbA1c starts to increase within the normal reference range and the rise is steepest during the last 6 months before diagnosis. The strength of our study is the fact that with a considerably larger sample size and higher number of HbA1c measurements per child we were able to get more accurate and reliable estimates when analyzing the predictive characteristics of HbA1c. Our study population came from three clinical centers that used different methods to analyze HbA1c, which might have produced slightly different absolute values. This was taken into account using covariate adjustment in the analyses. Furthermore, we used a relative increase in HbA1c rather than absolute values in our predictive model to make the results of our study more generalizable. Currently there are no exact threshold values for a significant change in HbA1c levels. Our decision rules were based on available literature and variances reported by the university hospital laboratories involved, and they are described in more detail in the Research Design and Methods section.

The suggested decision rules of a 10% rise in HbA1c during 3-12 months and two consecutive
HbA1c values ≥5.9% (41 mmol/mol) both provide high hazard ratios with relatively short median time to diagnosis of type 1 diabetes (1.1 and 0.9 years, respectively). However, a 10% rise provides superior sensitivity (57% vs. 42%), whereas two consecutive results of ≥5.9% (41 mmol/mol) has higher specificity (86% vs. 66%). Since children with multiple (≥2) autoantibodies have a high risk of developing type 1 diabetes over a variable period of time it is probably more practical from the clinical point of view to use two consecutive HbA1c results ≥5.9% in the prediction of timing of diagnosis. It is still noteworthy that a relative increase of 10% in HbA1c is more independent from methodological differences and reference values, remaining therefore important alongside the absolute values.

Our retrospective analysis showed that HbA1c values start to increase around 2 years before the diagnosis reflecting the gradual deterioration in endogenous insulin secretion and increasing fluctuation in plasma glucose levels. There were some differences in the demographic characteristics between the non-progressors and the progressors, however. The non-progressors were older both at initial seroconversion and when developing multiple autoantibodies. The mean age of the non-progressors was 10.9 years at the end of follow-up, whereas the age of the progressors at diagnosis was 6.3 years. The non-progressors had less often affected family members. On the other hand, our data show that the mean HbA1c level remained very stable in the children in the non-progressor group during the follow-up, varying from 5.3% (34 mmol/mol) to 5.6% (38 mmol/mol), and therefore the differences in age and follow-up time are probably not interfering with this analysis.

Our results provide important data on the natural evolution of HbA1c during the prediabetic period in young children, which can be utilized in future prevention trials. In secondary prevention trials aimed at slowing down or reversing the progression of β-cell dysfunction in subjects with islet autoantibodies there is a clear need of markers that can be used to monitor the disease process. Still
HbA1c is an indirect measure of glucose control and cannot be used as a direct estimate of β-cell function. It is noteworthy that the time interval between the HbA1c measurements used to calculate the presence or not of a 10% increase varied between 3 to 12 months and may represent a potential confounder, given that a 10% increase occurring during a 3 month-interval might have a different weight in terms of prediction compared to a similar increase in samples derived from a 12-month interval. As HbA1c represents variation in plasma glucose levels during the lifetime of erythrocytes (120 days) with the weight on the preceding 6-8 weeks, it seems unnecessary to measure HbA1c more often than every 3 months. Since the three laboratories involved reported rather high uncertainty of measurements varying from 5.8% to 10% and the methodology changed over the study period one have to consider the possibility that some changes in HbA1c levels are due to variations in methodology. However, this possible source of error affects equally the progressor and non-progressor groups. The HbA1c values suggested on the basis of the current analyses for the prediction of clinical type 1 diabetes are from a single study and validation of the results is needed in additional populations.

In conclusion, we have shown that HbA1c is a useful biochemical marker for the estimation of the time to diagnosis of type 1 diabetes in genetically susceptible children with multiple islet autoantibodies.
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Author Contributions: O.H. and R.V. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. O.H. S.A. contributed equally as first authors.

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Critical revision of the manuscript for important intellectual content: O.H., S.A., T.P., M.-R.H., N.H., J.L., J.I., M.K., R.V.

Statistical analysis: T.P.

Administrative, technical, or material support: O.H., T.P., J.L., J.I., M.K., R.V.

Study supervision: M.K., R.V.

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LEGENDS FOR THE FIGURES

**Figure 1** - Adjusted mean HbA1c values during follow-up in children with multiple islet autoantibodies. In the linear mixed model analysis HbA1c values were adjusted for gender, age at sampling, age at seroconversion, age at multiple (≥2) autoantibody positivity, type 1 diabetes in a first degree relative, and HLA risk. The last points are from the diagnosis of type 1 diabetes (progressors, black squares) or the last follow-up visit by December 31, 2011 (non-progressors, open squares). Whiskers show 95% confidence intervals of adjusted mean. HbA1c started to be significantly and consistently higher in the progressors 2.0 years before diagnosis. The number of subjects at each time point is shown at the bottom of the figure.

**Figure 2** - Cox regression estimates showing time to diagnosis of type 1 diabetes from the time when multiple autoantibodies were detected among children with multiple islet autoantibodies. Median survival time is indicated (Md, years). The four panels show survival curves for (A) all children with multiple (≥2) islet autoantibodies, (B) children with/without a 10% increase in HbA1c during 3-12 months or not, (C) children with/without a 10% increase in HbA1c during 3-12 months and any additional increase in HbA1c during the next 6 months, or not, and (D) children with/without two consecutive HbA1c values ≥5.9% (41 mmol/mol) or not.
Table 1 - Baseline characteristics of the study population.

<table>
<thead>
<tr>
<th></th>
<th>Progressors (^i)</th>
<th>Non-progressors (^d)</th>
<th>All (N=466)</th>
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</thead>
<tbody>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>123 (61)</td>
<td>166 (63)</td>
<td>289 (62)</td>
</tr>
<tr>
<td>Girls</td>
<td>78 (39)</td>
<td>99 (37)</td>
<td>177 (38)</td>
</tr>
<tr>
<td>T1D(^i) in first degree relative at the time of birth, n (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>No</td>
<td>168 (84)</td>
<td>245 (92)</td>
<td>413 (89)</td>
</tr>
<tr>
<td>Yes</td>
<td>33 (16)</td>
<td>20 (8)</td>
<td>53 (11)</td>
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<tr>
<td>HLA risk(^d), n (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Low</td>
<td>23 (11)</td>
<td>33 (13)</td>
<td>56 (12)</td>
</tr>
<tr>
<td>Moderate</td>
<td>122 (61)</td>
<td>178 (67)</td>
<td>300 (65)</td>
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<tr>
<td>High</td>
<td>56 (28)</td>
<td>53 (20)</td>
<td>109 (23)</td>
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<tr>
<td>Age (years) at seroconversion, mean (SD)</td>
<td>2.5 (2.0)</td>
<td>3.9 (2.8)</td>
<td>3.3 (2.6)</td>
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<tr>
<td>Age (years) at multiple (≥2) autoantibodies, mean (SD)</td>
<td>3.0 (2.1)</td>
<td>5.2 (3.4)</td>
<td>4.2 (3.1)</td>
</tr>
<tr>
<td>Age (years) at T1D diagnosis or age of non-progressors at last measurement(^5), mean (SD)</td>
<td>6.3 (3.3)</td>
<td>10.9 (4.1)</td>
<td>8.9 (4.4)</td>
</tr>
<tr>
<td>Follow-up time (years), mean (SD)</td>
<td>3.4 (2.6)</td>
<td>5.6 (3.5)</td>
<td>4.7 (3.3)</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
</tbody>
</table>

1. Progressors are children with multiple (≥2) autoantibodies who progressed to overt type 1 diabetes.
2. Non-progressors had multiple autoantibodies but did not develop clinical disease during the follow-up.
3. T1D denotes type 1 diabetes.
4. One non-progression possessed a rare HLA genotype not possible to determine.
5. The follow-up ended at 12/31/2011.
Table 2 - Univariate Cox regression hazard ratios (HR) for the contribution of clinical factors to the progression of type 1 diabetes (T1D).

<table>
<thead>
<tr>
<th>Clinical Factor</th>
<th>Progressors</th>
<th>Non-progressors</th>
<th>HR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate cox regression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% increase in HbA1c values within 3-12 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No, n (%)</td>
<td>87 (43)</td>
<td>175 (66)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>114 (57)</td>
<td>90 (34)</td>
<td>5.7</td>
<td>4.1 to 7.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10% increase in HbA1c values within 3-12 months and any additional increase during the next 6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No, n (%)</td>
<td>156 (78)</td>
<td>242 (91)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>45 (22)</td>
<td>23 (9)</td>
<td>5.1</td>
<td>3.6 to 7.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Two consecutive HbA1c values ≥ 5.9% (41 mmol/mol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No, n (%)</td>
<td>116 (58)</td>
<td>229 (86)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>85 (42)</td>
<td>36 (14)</td>
<td>11.9</td>
<td>8.8 to 16.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (years) at multiple (≥2) autoantibodies, mean (SD)</td>
<td>3.0 (2.1)</td>
<td>5.2 (3.4)</td>
<td>0.8</td>
<td>0.8 to 0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time (years) from seroconversion to multiple (≥2) autoantibodies, mean (SD)</td>
<td>0.4 (1.1)</td>
<td>1.3 (2.1)</td>
<td>0.7</td>
<td>0.6 to 0.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
T1D in first degree relative at the time of birth

<table>
<thead>
<tr>
<th></th>
<th>Study 1</th>
<th>Study 2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No, n (%)</td>
<td>168 (84)</td>
<td>245 (92)</td>
<td>1</td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>33 (16)</td>
<td>20 (8)</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.3 to 2.7</td>
</tr>
</tbody>
</table>

HLA risk

<table>
<thead>
<tr>
<th></th>
<th>Study 1</th>
<th>Study 2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low, n (%)</td>
<td>23 (11)</td>
<td>33 (13)</td>
<td>1</td>
</tr>
<tr>
<td>Moderate, n (%)</td>
<td>122 (61)</td>
<td>178 (67)</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.6 to 1.5</td>
</tr>
<tr>
<td>High, n (%)</td>
<td>56 (28)</td>
<td>53 (20)</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.8 to 2.1</td>
</tr>
</tbody>
</table>

Gender

<table>
<thead>
<tr>
<th></th>
<th>Study 1</th>
<th>Study 2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys, n (%)</td>
<td>123 (61)</td>
<td>166 (63)</td>
<td>1</td>
</tr>
<tr>
<td>Girls, n (%)</td>
<td>78 (39)</td>
<td>99 (37)</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.8 to 1.5</td>
</tr>
</tbody>
</table>

1 Progressors are children with multiple (≥2) autoantibodies who progressed to overt type 1 diabetes.

2 Non-progressors had multiple autoantibodies but did not develop clinical disease during the follow-up.

3 One non-progressor possessed a rare HLA genotype not possible to determine.
### Table 3 - Multivariate Cox regression adjusted hazard ratios (HR) for the contribution of clinical factors to progression of type 1 diabetes (T1D) after controlling for other variables.

<table>
<thead>
<tr>
<th></th>
<th>Progressors N=201</th>
<th>Non-progressors N=265</th>
<th>Adjusted</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multivariate Cox regression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% increase in HbA1c values within 3-12 months, n (%)</td>
<td>114 (57)</td>
<td>90 (34)</td>
<td>2.8</td>
<td>2.0 to 4.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Two consecutive HbA1c values ≥ 5.9% (41 mmol/mol), n (%)</td>
<td>85 (42)</td>
<td>36 (14)</td>
<td>8.5</td>
<td>6.1 to 11.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (years) at multiple (≥2) autoantibodies, mean (SD)</td>
<td>3.0 (2.1)</td>
<td>5.2 (3.4)</td>
<td>0.8</td>
<td>0.7 to 0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High HLA risk, n (%)</td>
<td>56 (28)</td>
<td>53 (20)</td>
<td>1.7</td>
<td>1.2 to 2.2</td>
<td>0.001</td>
</tr>
<tr>
<td>T1D in first degree relative at the time of birth, n (%)</td>
<td>33 (16)</td>
<td>20 (8)</td>
<td>1.5</td>
<td>1.0 to 2.3</td>
<td>0.043</td>
</tr>
</tbody>
</table>

1 Progressors are children with multiple (≥2) autoantibodies who progressed to overt type 1 diabetes.

2 Non-progressors had multiple autoantibodies but did not develop clinical disease during the follow-up.

3 Explanatory variable candidates were a 10% increase in HbA1c values within 3-12 months, a 10% increase in HbA1c values within 3-12 months and any additional increase during the next 6 months, two consecutive HbA1c values ≥ 5.9% (41 mmol/mol), age at multiple (≥2) autoantibodies, T1D in first degree relative, HLA risk and gender.
| Time   | -7 | -6.5 | -6 | -5.5 | -5 | -4.5 | -4 | -3.5 | -3 | -2.5 | -2 | -1.8 | -1.6 | -1.4 | -1.2 | -1.0 | -0.8 | -0.6 | -0.4 | -0.2 | 0  |
|--------|----|------|----|------|----|------|----|------|----|------|----|------|-----|------|-----|------|-----|------|-----|------|----|------|
| Progressors | 8  | 13   | 14 | 18   | 25 | 31   | 40 | 53   | 61 | 76   | 63 | 52   | 71  | 81   | 80  | 80   | 83  | 101  | 111 | 108  | 148 |
| Non-Progressors | 29 | 35   | 41 | 51   | 71 | 75   | 90 | 99   | 110| 122  | 82 | 96   | 101 | 94   | 99  | 120  | 122 | 155  | 148 | 11   | 265 |