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Predictors of Slow Progression to Diabetes in Children with Multiple Islet Autoantibodies

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Abstract

Although most children with multiple islet autoantibodies develop type 1 diabetes, rate of progression is highly variable. The goal of this study was to explore potential factors involved in rate of progression to diabetes in children with multiple islet autoantibodies. The Diabetes Autoimmunity Study in the Young (DAISY) has followed 118 children with multiple islet autoantibodies for progression to diabetes. After excluding 27 children currently diabetes-free but followed for <10 years, the study population was grouped into: rapid progressors (N=39) who developed diabetes in <5 years; moderate progressors (N=25), diagnosed with diabetes within 5–10 years; and slow progressors (N=27), diabetes-free for >10 years. Islet autoimmunity appeared at 4.0 ± 3.5 , 3.2 ± 1.8 and 5.8 ± 3.1 years of age in rapid, moderate and slow progressors, respectively ($p=0.006$). Insulin autoantibody levels were lower in slow progressors compared to moderate and rapid progressors. The groups did not differ by gender, ethnicity, family history, susceptibility HLA and non-HLA genes. The rate of development of individual islet autoantibodies including mIAA, GADA, IA-2A and ZnT8A were all slower in the slow versus moderate/rapid progressors. In multivariate analyses, older age at seroconversion and lower initial mIAA levels independently predicted slower progression to diabetes. Later onset of islet autoimmunity and lower autoantibody levels predicted slower progression to diabetes among children with multiple islet autoantibodies. These factors may need to be considered in the design of trials to prevent type 1 diabetes.

Keywords

multiple islet autoantibodies; islet autoimmunity; rate of progression to diabetes; type 1 diabetes; predictors for diabetes

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Conflict of Interest

The authors have no relevant conflict of interest to disclose.

1. Introduction

Prevention trials would benefit from a more precise method to predict overall staging and rate of progression to type 1 diabetes. Though genetic markers can identify varying risk, it is only once islet autoimmunity has begun (marked by the presence of multiple islet autoantibodies) that a high positive predictive value (>90%) can be achieved. Multiple islet autoantibodies are present in the great majority of prediabetics (1-3). Screening for risk of type 1 diabetes utilizes “biochemical” autoantibody assays for specific islet autoantigens (4). These include autoantibodies to insulin (mIAA) (5), glutamic acid decarboxylase (GADA) (6), IA-2A (ICA512) (7) and most recently ZnT8A (8). Individuals having a single positive autoantibody (mIAA, GADA, IA-2A or ZnT8A) are at low risk for progression to diabetes, while the majority of individuals expressing two or more positive autoantibodies, especially on multiple tests over time, will develop type 1 diabetes according to combined analysis from prospective birth cohort studies in Colorado, Finland, and Germany (9). However, rate of progression to diabetes among multiple autoantibody positive subjects varies widely, from a few months to several years after seroconversion.

Insulin autoantibodies are often high at the onset of diabetes in young children while usually negative in individuals first presenting with diabetes after age 12. High levels of insulin autoantibodies have been associated with younger age of onset of diabetes (10;11). In the BABYDIAB study, only non-HLA type 1 diabetes susceptibility genes but not autoantibody levels or HLA-DR3/4-DQ8 influenced rate of diabetes progression among first-degree relatives children with multiple islet autoantibodies (12).

In this study we evaluated potential factors involved in rate of progression to diabetes in multiple autoantibody positive subjects followed prospectively in the Diabetes Autoimmunity Study in the Young (DAISY). We found that later onset of islet autoimmunity and lower autoantibody levels predicted slower progression to diabetes among children with multiple islet autoantibodies.

2. Material and Methods

2.1. Study population

Since 1993, DAISY has followed two cohorts of young children at increased risk of type 1 diabetes (total N=2542): a cohort of relatives of type 1 diabetes patients (siblings and offspring), and the general population newborn cohort. The latter consists of children with type 1 diabetes susceptibility HLADR/DQ genotypes identified through screening of over 31,000 newborns at St. Joseph Hospital in Denver, Colorado. Recruitment began in 1993 and ended in 2004. The details of screening and follow-up have been previously published (13). Autoantibodies to insulin, GAD65, IA-2 and ZnT8 were measured in the Immunogenetic Laboratory at the Barbara Davis Center using previously described radio-immunoassays (14). Children in DAISY were tested for islet autoantibodies during the prospective follow-up, beginning at 9 months, 15 months, 24 months and annually thereafter; autoantibody positive children are followed and tested for islet autoantibodies every 3-6 months. Only DAISY children who have developed multiple (2 or more) islet autoantibodies (N=118) were included in this study. Three groups were defined according to time from

seroconversion: rapid progressors (N=39) who developed type 1 diabetes in <5 years, moderate (N=25) diagnosed with diabetes within 5-10 years and slow progressors (N=27) who had a diabetes-free follow-up for >10 years (5 eventually developed diabetes). Excluded were 27 children diabetes-free followed for <10 years. The cut-offs of >10 years and < 5 years were chosen to be consistent with other prospective studies and risk assessment estimated in these children (9;12;15). Onset of diabetes was defined according to ADA criteria. Informed consent was obtained from the parents of each study subject. The Colorado Multiple Institutional Review Board approved all study protocols.

2.2. Islet Autoantibodies

Measurement of islet autoantibodies to insulin, GAD65, IA-2 and ZnT8 was performed in the Clinical Immunology Laboratory at the Barbara Davis Center (BDC) using radio-immunoassays as described in the paper by Yu et al (16). In the 2015 IASP Workshop, sensitivities and specificities were 52% and 100% respectively for mIAA, 82% and 99% respectively for GADA, 72% and 100% respectively for IA-2A, and 70% and 97% respectively for ZnT8A. Measurement of electrochemiluminescence (ECL) assays was performed on yearly DAISY samples in the Immunogenetic Laboratory at the BDC using methods previously described (17;18). The ECL assay cut-off indexes of 0.006 for ECL-IAA or 0.023 for ECLGADA were set at the 99th percentile over 100 healthy controls and the ECL inter-assay co-efficiencies of variation (CV) were 4.8% (n=20) for ECL-IAA and 8.8% (n=10) for ECL-GADA, respectively. In the 2015 IASP Workshop, sensitivities and specificities for the ECL assays were 60% and 98% respectively for ECL-IAA, and 78% and 96% respectively for ECL-GADA, among patients with newly diagnosed T1D.

2.3. Genotyping

Non-HLA single nucleotide polymorphisms (SNPs) were genotyped using either a linear array (immobilized probe) method essentially as described in Mirel et al.(19), Illumina GoldenGate Beadexpress assays (veracode 48-plex) or Taqman SNP genotyping assays (Applied Biosystems, CA USA) as previously described (20). The 25 following non-HLA SNPs were analyzed: rs2292239 (*ERBB3*), rs12708716 (*CLEC16A*), rs4788084 (*IL27*), rs7202877 (*CTRB*), rs4900384 (*C14orf*), rs2290400 (*GSDM*), rs5753037 (*HORMAD2*), rs56297233 (*BACH2*), rs4763879 (*CD69*), rs7020673 (*GLIS3*), rs1990760 (*IFIH1*), rs3024496 (*IL10*), rs917997 (*IL18RAP*), rs12251307 (*IL2RA*), rs689 (*INS*), rs1893217 (*PTPN2*), rs2476601 (*PTPN22*), rs3184504 (*SH2B3*), rs2281808 (*SIRPG*), rs1738074 (*TAGAP*), rs11203203 (*UBASH3A*), rs9976767 (*UBASH3A*), rs13266634 (*SLC30A8*), rs231775 (*CTLA4*) and *CCR5* (microsatellite).

2.4. Statistical Analysis

Statistical analyses were performed using PRISM (GraphPad Software, Inc., La Jolla, CA) and SAS version 9.2 (SAS Institute, Cary, NC, USA). Autoantibody levels were converted to SD units away from threshold (Z scores) and then log transformed for analyses. Because of negative values, 1 was added before log transformation and calculation of mean. Proportions were compared using chi-square or Fisher's exact test. Follow-up time was defined as time from the initial positive autoantibody test for each subject. Time to diabetes was defined as the time from initial seroconversion. Multivariate time-varying Cox PH model was used to

evaluate potential factors involved in rate of progression to diabetes, including age at seroconversion, family history of type 1 diabetes, number of initial autoantibodies and time-varying autoantibody levels. To test the proportional hazards assumption, we used the supreme test methods. The proportional hazards assumption was tested for the following fixed variables, number of first positive autoantibodies, family history of type 1 diabetes and HLA DR3/4-DQ8. We concluded that the proportional hazards assumption was not violated. Survival analysis was performed for development of autoantibodies using the log-rank test.

3. Results

The characteristics of study participants are shown in Table 1. Gender, ethnicity, family history of type 1 diabetes and HLA DR3/4-DQ8 were not significantly different between the groups. Slow progressors had later onset of islet autoimmunity compared to moderate and rapid progressors (5.8 ± 3.1 , 3.2 ± 1.8 and 4.0 ± 3.5 years respectively, $p=0.006$). Slow progressors were also less likely to have ever been positive for ECL-IAA compared to moderate and rapid progressors (74%, 100% and 97% respectively, $p=0.001$), but no differences were seen for ECL-GADA.

Autoantibody levels for subjects who developed diabetes are summarized in Table 2. Slow progressors had lower initial mIAA levels than moderate and rapid progressors (-0.06 , -0.04 and 0.15 respectively, $p=0.015$). No differences were found for the other autoantibodies between the three groups.

Multivariate time-varying Cox PH model was performed to evaluate potential factors involved in rate of progression to diabetes, including age at seroconversion, family history of type 1 diabetes, HLA-DR3/4-DQ8, number of initial autoantibodies and time-varying autoantibody levels. Age at seroconversion (HR 0.8, 95% CI 0.7-1.0, $p=0.044$) and mIAA level (HR 1.1, 95% CI 1.0-1.2, $p=0.002$) were significant predictors of time to progression to type 1 diabetes in children who were multiple autoantibody positive (Table 3).

The rate of development of individual islet autoantibodies was slower in the slow versus moderate/rapid progressors (Figure 1). Slow progressors had a lower risk of developing mIAA at 10 years of age compared to moderate and rapid progressors (44%, 73% and 81% respectively, $p=0.007$) with similar results for IA-2A (44%, 76% and 72% respectively, $p=0.016$). Development of GADA at 10 years was also lower in slow progressors compared to moderate and rapid progressors (56%, 92% and 77% respectively, $p=0.008$) with similar results for ZnT8A (41%, 91% and 66% respectively, $p=0.0003$). In addition, rate of progression from single to multiple autoantibodies was slower in the slow versus moderate/rapid progressors (70% at 2 years since seroconversion vs 96% and 95% respectively, $p=0.01$) (Figure 2).

Non-HLA susceptibility polymorphisms previously genotyped in DAISY (N=25) were evaluated for association with progression to diabetes from islet autoimmunity (20). No significant differences were found between the three groups. When comparing the two groups of slow to rapid progressors, only rs4763879 minor allele A (*CD69*) was significantly less frequent in slow progressors ($p=0.036$). We combined 5 gene SNPs

available in 75 of the 91 subjects and previously associated with either islet autoimmunity and/or type 1 diabetes in DAISY into a genetic risk score. For all 5 gene SNPs, a score of 2 was given if the child was homozygous for the susceptible allele, 1 if heterozygous and 0 if homozygous for the non-susceptible allele. The sum of the scores for the 5 genes was assigned as the combined risk score for each child. The risk scores from all 5 SNPs (*PTPN22* rs2476601, *INS* rs689, *CTLA4* rs231775, *UBASH3A* rs11203203, *GLIS3* rs7020673) ranged from 2 to 8. The genetic risk score among the 3 groups was not significantly different ($p=0.99$) (data not shown).

4. Discussion

Whether being a first-degree relative with type 1 diabetes or not, children with two or more positive islet autoantibodies have a 70% risk for developing diabetes in ten years following appearance of autoantibodies (9). Prospective studies such as DAISY and TEDDY (The Environmental Determinants of Diabetes in the Young) have shown that mIAA and, in TEDDY IA-2A levels, (but not GADA levels) were useful in estimating the age at diabetes onset. Both nonfluctuating mIAA and mIAA levels were significant factors for progression to diabetes (11;15). DAISY has the longest follow-up of children at risk for diabetes coming from both the general population and relatives of type 1 diabetes patients. This study found that later onset of islet autoimmunity and lower autoantibody levels predicted slower progression to diabetes among children with multiple autoantibodies. In addition, the rate of development of individual islet autoantibodies including mIAA, GADA, IA-2A and ZnT8A as well as development of multiple autoantibodies were slower in the slow versus moderate/rapid progressors.

Factors involved in slow progression to diabetes once islet autoimmunity is confirmed are largely unknown, but would be helpful in both design of prevention trials and the understanding of type 1 diabetes pathophysiology. Results from the BABYDIAB study (12) did not show significant differences in islet autoantibody profiles between rapid and slow progressors, except for a delayed development of IA-2A in slow progressors. Similar to DAISY, this study did not find any differences in HLA DR/DQ risk categories nor in 13 non-HLA genes overall between the slow and rapid progressors. However, combined risk score from 7 non-HLA genotypes (*IL2* rs4505848, *CD25* rs11594656, *INS* VNTR, *IL18RAP* rs917997, *IL10* rs3024505, *IFIH1* rs2111485 and *PTPN22* rs6679677) was able to discriminate between rapid and slow progressors. The different results in non-HLA genes can be explained by the number and type of single nucleotide polymorphisms (SNPs) included in these two studies. In addition, BABYDIAB only follows relatives of patients with type 1 diabetes, while DAISY follows both general population children and relatives of type 1 diabetes patients.

The results of this study are consistent with previously known factors involved in high risk of development of diabetes. Age at seroconversion and autoantibody characteristics such as levels and affinity have been shown to increase risk of progression to diabetes (11;15;21;22). In addition, the presence of autoantibodies that typically develop later such as IA-2A and ZnT8A has been described as an increased risk for development of diabetes (23;24). In TrialNet, an autoantibody risk score (ABRS) (25) was developed from a proportional

hazards model that combined autoantibody levels from each autoantibody (GADA, IA-2A, mIAA, ICA and ZnT8A). The ABRS was strongly predictive of diabetes in the TrialNet population. The same factors involved in risk for development of type 1 diabetes seem to be important in the rate of progression to diabetes as well. An inverse log-linear relationship between levels of insulin autoantibodies and time of progression from first appearance of islet autoantibodies to diagnosis of diabetes has been previously reported in prospectively followed DAISY children (11), and IAA levels were also significant factors for progression to diabetes in the TEDDY cohort (15). Levels of insulin autoantibodies may therefore be a marker of rate of the disease process. Although the underlying mechanism is unknown, there is some evidence that proinsulin/insulin molecules are a primary target of the islet autoimmunity resulting in diabetes of the non-obese diabetic (NOD) mouse with anti-insulin T cell autoimmunity the primary driver of beta cell destruction (26).

The limitations of this study include small numbers of subjects in each group as well as incomplete non-HLA type 1 diabetes-susceptibility SNP information. Other factors such as OGTT and DPT-1 risk score (risk score based on age, BMI and OGTT indexes) could not be analyzed because of small numbers (27) (28).

Electrochemiluminescence (ECL) assays have been shown to be more disease specific and, for ECL-IAA more sensitive, than radioassays (17;18;29). Slow progressors were less likely to have been positive for ECL-IAA, but no differences were seen for ECL-GADA. ECL assays were not included in the multivariate time-varying Cox PH model as these assays were measured yearly in DAISY and not at every visit. The higher disease specificity of ECL assays seems to be related to higher affinity, confirming that autoantibody characteristics are likely important contributors to rate of progression to diabetes.

Factors involved in slower progression to diabetes among children with multiple autoantibodies include age at seroconversion and autoantibody characteristics. These factors may need to be considered in the design of trials to prevent type 1 diabetes.

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Highlights

- In subjects with multiple islet autoantibodies, insulin autoantibody levels are lower in slow progressors compared to moderate and rapid progressors
- Onset of islet autoimmunity happens later in slow progressors
- Rate of development of individual islet autoantibodies (mIAA, GADA, IA-2A and ZnT8A) are slower in the slow versus moderate/rapid progressors
- Rate of progression from single to multiple autoantibodies is slower in the slow versus moderate/rapid progressors
- No significant differences in HLA susceptibility genotypes between slow versus moderate/rapid progressors

Figure 1.

Development of individual autoantibodies by groups (since birth)

Development of mIAA, GADA, IA-2A and ZnT8A since birth in slow, moderate and rapid progressors.

Dev.: development

Mod: moderate

Figure 2.
Development of multiple (>2) autoantibodies by groups (since seroconversion)
Development of multiple (>2) autoantibodies since seroconversion in slow, moderate and rapid progressors.
Dev.: development
Ab: autoantibodies
Mod: moderate

Table 1

Characteristics of slow, moderate and rapid progressors in DAISY multiple autoantibody positive subjects

Characteristics	Slow Progressors (N=27)	Moderate Progressors (N=25)	Rapid Progressors (N=39)	P value ^a
Male Gender, N (%)	17 (63)	10 (40)	22 (56)	0.23
Ethnicity NHW, N (%)	21 (78)	23 (92)	36 (92)	0.19
FDR ^b with type 1 diabetes	19 (70)	14 (56)	26 (67)	0.53
HLA DR3/3, N (%)	0 (0)	0 (0)	2 (5)	0.38
HLA DR3/4-DQ8, N (%)	11 (41)	10 (40)	24 (62)	
HLA DR4/4, N (%)	2 (7)	3 (12)	4 (10)	
HLA DR3/X, N (%)	7 (26)	4 (16)	4 (10)	
HLA DR4/X, N (%)	4 (15)	6 (24)	4 (10)	
HLA DRX/X, N (%)	3 (11)	2 (8)	1 (3)	
ECL-IAA case ^c , N (%)	20 (74)	25 (100)	38 (97)	0.001
ECL-GADA case ^c , N (%)	19 (70)	20 (80)	29 (74)	0.73
Initial radioassay Ab ^d count ^e	1.5 ± 0.7	1.7 ± 0.7	1.8 ± 1.0	0.40
Age at seroconversion ^e	5.8 ± 3.1	3.2 ± 1.8	4.0 ± 3.5	0.006
Follow-up ^f from seroconversion ^e	13.2 ± 2.6	7.2 ± 1.5	2.7 ± 1.5	<0.0001
Follow-up ^f from birth ^e	19.0 ± 3.6	10.4 ± 2.0	6.7 ± 4.0	<0.0001

^aP value: chi-square or Fisher exact test for categorical variables, ANOVA for continuous variables^bFDR: first-degree relative^cECL case: has ever been positive for ECL^dAb: autoantibodies^eMean ± SD^fFollow-up: follow-up to development of diabetes or last visit for those slow progressors who haven't developed diabetes

Table 2

Autoantibody characteristics in DAISY multiple autoantibody positive subjects

Variable	Slow progressors (N=5) ^a	Moderate progressors (N=25)	Rapid progressors (N=39)	P value
Initial mIAA ^b	-0.06 ± 0.13	-0.04 ± 0.12	0.15 ± 0.34	0.015
Initial ECL-IAA ^b	-0.06 ± 0.15	-0.08 ± 0.14	0.04 ± 0.28	0.14
Initial GADA	-0.14 ± 0.43	-0.04 ± 0.34	-0.17 ± 0.32	0.33
Initial ECL-GADA ^b	-0.08 ± 0.22	-0.08 ± 0.19	-0.04 ± 0.26	0.82
Initial IA-2A	0.20 ± 0.09	0.18 ± 0.12	0.24 ± 0.19	0.44
Initial ZnT8A ^b	-0.02 ± 0.01	-0.02 ± 0.02	-0.01 ± 0.03	0.90

Data is mean ± SD. Autoantibody levels were converted to SD units away from threshold (Z scores). Because of negative values, 1 was added before log transformation and calculation of mean.

^a only subjects who developed diabetes are included in these analyses

^b missing information in some subjects: ECL is measured yearly, not at every visit

Table 3

Multivariate time-varying Cox PH model (Analysis of Maximum Likelihood Estimates)

Parameter	Hazard Ratio (95% CI)	P value
FDR ^a with type 1 diabetes	0.42 (0.15-1.20)	0.105
HLA DR3/4-DQ8	1.91 (0.78-4.68)	0.160
Age at seroconversion	0.82 (0.67-1.00)	0.044
Initial radioassay Ab ^b count	1.72 (0.98-3.0)	0.057
mIAA	1.12 (1.04-1.21)	0.002
GADA	0.95 (0.88-1.02)	0.124
IA-2A	1.03 (0.94-1.13)	0.519
ZnT8A	1.38 (0.76-2.50)	0.287

Multivariate models including age at seroconversion, family history of type 1 diabetes, HLA-DR3/4-DQ8, number of initial radioassay autoantibodies and time-varying autoantibody levels. Time to diabetes was defined as the time from initial seroconversion. ECL assays were not included in these models as these assays are measured yearly and not at every visit.

^aFDR: first-degree relative

^bAb: autoantibodies