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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.

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

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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 HLA-DR/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 ECL-GADA were set at the 99th percentile over 100 healthy controls and the ECL inter-assay coefficiencies 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 (Fig. 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

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