Islet Autoantibody Seroconversion in the DPT-1 Study

Justification for repeat screening throughout childhood

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OBJECTIVE—Although type 1 diabetes autoimmunity frequently begins in childhood, little is known about the relationship between age and autoimmunity development. Our aim was to determine the timing of seroconversion to diabetes-associated autoantibody (DAA) positivity and risk in first- and second-degree relatives of patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS—Study subjects were identified through the Diabetes Prevention Trial-Type 1 (DPT-1). Children 3–18 years of age (n = 42,447) were screened for DAAs; 1,454 were ICA positive (≥10 JDF units), 1,758 were GAD65 positive, and 899 were ICA512 positive at the time of initial screening. Subjects who were initially antibody negative (n = 39,212) were recalled for rescreening, and 11,813 returned for rescreening.

RESULTS—DAA seroconversion occurred in 469 (4%) children; 258 seroconverted to ICA, 234 to GAD65, and 99 to ICA512. The median time to seroconversion was 2 years. The 2-year risk for DAAs was highest in early childhood. For each 1-year increase in age in this cohort, the risk of any autoantibody seroconversion (HR 0.95, 95% CI 0.92–0.97) decreased by 5%, and for any two autoantibodies risk decreased by 13% (0.87, 0.82–0.93).

CONCLUSIONS—Risk of autoantibody seroconversion among children followed in DPT-1 is age dependent. Younger children have the highest risk for DAAs, with the majority of children seroconverting by 13 years of age (75%). This suggests that annual screenings should be started in early childhood and continued through early adolescence to identify the majority of subjects at risk for type 1 diabetes and eligible for prevention trials.

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It is well known that the presence of islet autoantibodies increases the risk of type 1 diabetes (1,2). The known diabetes-associated autoantibodies (DAAs) associated with type 1 diabetes include cytoplasmic islet cell (ICA), insulin (IAA, microIAA [mIAA]), 65-kDa isoform of glutamic acid decarboxylase (GAD-65, GADA, GAA), and insulinoma-associated protein 2 (IA-2, IA-2A, ICA512). These autoantibodies usually precede type 1 diabetes onset and are regularly used as indicators to identify the preclinical period of the disease. Autoimmunity screening for DAAs is useful in identifying individuals with increased risk of type 1 diabetes (1,3).

A limited number of previous studies have suggested the need for autoantibody rescreening but have been limited in their ability to extensively describe the natural history of seroconversion throughout childhood. Colman et al. (4) demonstrated that 3.4% of children with an affected first-degree relative who screened negative for islet autoantibodies in early childhood (<8 years of age) later screened positive at a mean age of 11 years; thus, reliance on a single screening effort in early childhood would have failed to identify a considerable portion of genetically at-risk children who seroconverted after 8 years of age. They concluded that screening should be performed more than once before puberty.

The importance of understanding the relationship between age and the onset of autoimmunity provides valuable insight regarding preclinical type 1 diabetes screening; first, to determine if there is an optimal age window(s) to screen for autoantibodies; second, to determine the timing of the onset of autoimmunity relative to environmental exposures so as to potentially identify mechanisms involved in the disease; and, third, to identify patients for prevention trials. This work assessed the characteristics of DAAs, yield of screening efforts, and the risk of first autoantibody development and multiple autoantibody development by age.

RESEARCH DESIGN AND METHODS

—All subjects were participants in the screening cohort of the DPT-1 study (5) who were screened for the presence of ICAs between February 1994 and October 2002 for potential study accrual to delay or prevent type 1 diabetes. The current study group was comprised of first- and second-degree relatives of patients with type 1 diabetes (3–18 years of age) who screened negative for all DAAs (ICA, ICA512, GAD65, and mIAA) at their first screening and returned for a rescreening. mIAA was measured on all subjects at their initial screening and used as inclusionary criteria to determine autoantibody-free status; thereafter, mIAA was only measured on a small proportion of the DAA-negative subjects at rescreening. Thus, this study did not assess risk for mIAA. Autoantibody-negative subjects were rescreened at subsequent visits for ICA, ICA512, and GAD65. For all other DAAs, those aged 3–10 years were rescreened annually, and those aged >10 years were screened biennially. Approximately 30% of this population had at least one or more autoantibody rescreening.

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Autoantibody assays

ICA values were determined using the standard indirect immunofluorescence method using cryo-cut sections of frozen sections of human pancreas at the DPT-1 ICA core laboratory (Gainesville, FL, and New Orleans, LA). Titers ≥10 Juvenile Diabetes Foundation units (JDFU) were considered positive for ICA. The ICA assays had a specificity of 100% with a sensitivity of 74.4% (6).

GAD65, ICA512, and mIAA

GAD65 and ICA512 were determined at the Barbara Davis Center in Denver, Colorado, and measured simultaneously by combined GAD65 and ICA512 radioassay as previously described (full-length GAD65 and ICA512bdc cDNA clones) (7). The assay was performed in 96-well filtration plates with autoantibody-bound [^{3}H]GAD65 and [^{35}S]ICA512 precipitated with protein A-Sepharose. The cut points were set at indices of ≥0.032 (GAD65) and ≥0.049 (ICA512A). The interassay coefficients of variation (CV) were 6.5% and 9.6%, respectively. This assay had a specificity of 99% and a sensitivity of 83.7% in subjects ≤30 years of age with new-onset type 1 diabetes in the IDS Combinatorial Autoantibody Workshop (6). IAA values, using the microvolume requiring assay, were determined at the Barbara Davis Center or the Joslin Diabetes Center (Boston, MA) (8). The sensitivity for the mIAA assay was 74%/56%, and the specificity was 90%/98% for the Denver and Boston laboratories. The correlation coefficient between both laboratories was $r = 0.90$, $P < 0.0001$, and the interassay CV was 16%.

The protocol was approved by the institutional review boards at participating locations, and all participants provided written informed consent.

Statistical analysis

Data were analyzed using the SAS software (version 9.2; SAS Institute, Cary, NC). Categorical variables were analyzed using Pearson χ^2 tests. Continuous variables were tested using *t* test for differences in means or Wilcoxon rank-sum test for differences in medians. Autoantibody seroconversion was defined as the first positive sample for a specific autoantibody. Cox proportional hazards models for an open cohort design were used to estimate 2-year risks for seroconversion and to determine significant predictors of seroconversion. Two-year risk estimates were chosen based on median follow-up time in this cohort. Efron's method for tied survival times were used in the Cox analysis (9). Selection of variables for the final model was accomplished by removing nonsignificant effects in a stepwise fashion. Entry and exit levels were at 5%. The assumption of constant hazard function was assessed by examining the log cumulative survival plots.

RESULTS—Characteristics of the study population by DAA development are presented in Table 1. Of the 42,447 subjects aged 3–18 years screened for DAAs in the DPT-1 Study, 3,235 subjects (7.6%) were autoantibody positive at the time of initial screening. Of these, (Table 2, first column) 1,454 subjects (3.4%) were ICA positive, 1,758 (4.1%) were GAD65 positive, 899 (2.1%) were ICA512 positive, and 1,094 (2.6%) were mIAA positive. At the initial screen, 39,212 subjects were autoantibody negative. There was no significant difference in age ($P = 0.2964$) or race/ethnicity ($P = 0.0798$) between the two groups (DAA+ vs. DAA−). There were small proportional differences between sexes ($P = 0.008$) (Table 1), and there was a significantly higher proportion of siblings of patients with type 1 diabetes ($P < 0.0001$) in children positive for an autoantibody.

For those DAA negative at the initial screen and who returned for at least one rescreening ($n = 11,813$), 469 (4.0%) seroconverted to DAA(s) positive, 258 (2.2%) to ICA positive, 234 (2.0%) to GAD65 positive, and 99 (0.8%) to ICA512 positive (Table 2). These children were significantly younger ($P < 0.0001$) in median age (years [quartile 1–quartile 3]) at the initial screen (7.1 years [5.1–10.0] vs. 8.2 years [5.5–11.6]) compared with the remaining children who did not seroconvert ($n = 11,344$). Median (Q1–Q3) time of follow-up for those who returned for a rescreening in the study was 27.1 months (20.1–49.1). There were no differences in race ($P = 0.39$), relation to proband ($P = 0.11$), or sex ($P = 0.42$) among those who seroconverted and those that remained autoantibody negative throughout follow-up. Fifty-four percent ($n = 251$) of the children who seroconverted did so by 11 years of age, with 75% seroconversion by 13 years of age. There was no significant difference in the number of rescreens for those who seroconverted ≤10 versus >10 years of age ($P = 0.76$).

DAAs had varying patterns of presentation in those children who seroconverted. Median ages at seroconversion (years [Q1–Q3]) were similar with the exception that children seroconverted to GAD65 at a significantly younger age than to ICA ($P = 0.02$) (GAD65, 9.5 years [7.1–12.6]; ICA, 10.2 years [8.2–13.7]; and ICA512, 9.9 years [7.3–13.5]). For those individuals who developed multiple autoantibodies, GAD65 seroconversion generally occurred earlier than ICA or ICA512, with ICA seroconversion usually occurring after ICA512. If a child tested positive for two DAAs at the same testing, it was likely a combination of GAD65 and ICA512, as the timing of each of these DAAs generally preceded the occurrence of ICA, giving rise to two positive autoantibodies.

Table 1—*Characteristics of the screened population aged 3–18 years*

	Ab− at initial screening	Ab+ at initial screening	*P*
Total (*N*)	39,212	3,235	
Age at initial screen (years)	9.7 (6.3–13.2)	9.8 (6.6–13.2)	0.2964
Sex (% female)	47%	49%	0.0079
Race (%)			0.0798
White	82	84	
Black	3	2	
Hispanic	12	11	
Other	1	1	
Unknown	2	2	
Relationship to index patient with type 1 diabetes (%)			<0.0001
Sibling	45	52	
Offspring	28	27	
Second-degree relative	27	21	

Data are median (Q1–Q3) and percent unless otherwise indicated.

Table 2—*Number of subjects autoantibody positive and screened/rescreened by specific autoantibody*

	Screen 1 (initial)	Rescreening: number autoantibody positive/number screened (%)							Total seroconversions/ rescreens
		2	3	4	5	6	7	8	
ICA	1,454/42,447 (3.4)	151/11,813 (1.3)	47/5,306 (0.9)	42/2,532 (1.7)	7/1,165 (0.6)	10/564 (1.8)	1/220 (0.5)	0/71 (0)	258/21,671 (1.2)
GAD65*	1,758/42,447 (4.1)	134/11,522 (1.2)	58/5,126 (1.1)	23/2,410 (1.0)	11/1,118 (1.0)	2/532 (0.4)	4/212 (1.9)	1/101 (1.0)	234/21,021 (1.1)
ICA512*	899/42,447 (2.1)	54/11,614 (0.5)	15/5,243 (0.3)	14/2,538 (0.6)	6/1,188 (0.5)	5/585 (0.9)	1/240 (0.4)	2/151 (1.3)	99/21,559 (0.5)
mIAA†	1,094/42,447 (2.6)								

Data are *N* (%). *GAD65 and ICA512 were measured on 98% of those that returned for a rescreening. †Rescreening not evaluated in this study.

The 2-year risk for seroconversion by age showed significant decreases ($P < 0.001$) with increasing age for all DAAs (Fig. 1). GAD65 2-year risk had a significantly different slope of decline compared with ICA512 ($P < 0.0001$) and ICA ($P < 0.0001$) across all ages, with GAD65 risk having a steeper slope for those aged 6 years and under. ICA risk was significantly higher than ICA512 risk for all ages ($P = 0.003$). The 2-year risk for any DAA was significantly ($P < 0.0001$) higher across all ages than for any two DAAs.

Using Cox proportional hazards models, the age at initial screening was identified as a significant predictor for risk of autoantibody seroconversion for all autoantibodies (ICA, $P = 0.01$; ICA512, $P = 0.004$; GAD65, $P < 0.0001$). In univariate models, siblings had a 46% (HR 1.46, 95% CI 1.06–2.03) greater risk of GAD65 seroconversion ($P = 0.02$), and females had 21% (0.79, 0.64–0.98) lower risk of ICA seroconversion ($P = 0.03$). Race/ethnicity was not a significant predicator for autoantibody seroconversion in this cohort. In multivariable models adjusted for race, sex, relation to index proband, and rescreen interval, age at initial screening was the primary predicator of autoantibody seroconversion for any DAA ($P < 0.0001$). The risk of DAA seroconversion greatly increased if the subject was already positive for another autoantibody. DAA seroconversion risk significantly decreased with increasing age for GAD65 (HR 0.90, 95% CI 0.87–0.93, $P < 0.0001$), ICA (0.96, 0.93–0.99, $P = 0.01$), and ICA512 (0.92, 0.87–0.98, $P = 0.004$). For each 1-year increase in age in this cohort, the risk of any autoantibody seroconversion (0.95, 0.92–0.97, $P < 0.0001$) decreased by 5% and for two autoantibodies risk decreased by 13% (0.87, 0.82–0.93, $P < 0.0001$).

Lastly, 69 (15%) of the 469 that seroconverted during rescreening developed multiple autoantibodies. Of the 69, 45 (65%) seroconverted to multiple autoantibodies at the same screening and 24 (35%) developed multiple autoantibodies over time. Twenty-four of the 469 subjects (5%) who DAA seroconverted developed type 1 diabetes. At the time of diagnosis, 8% were ICA positive only, 4% were GAD65 positive only, 4% were GAD65 and ICA512 positive, 17% were ICA and GAD65 positive, 21% were ICA512 and ICA positive, and 45% were positive for all three autoantibodies. There were 19 subjects that developed type 1 diabetes who had no autoantibodies at the last screening before diagnosis (median was 15 months prior to diagnosis). The median age (Q1–Q3) at type 1 diabetes diagnosis was 12.4 years (9.7–14.7).

CONCLUSIONS—Autoantibody seroconversion rates are age-dependent among the first- and second-degree relatives of type 1 diabetic probands followed through childhood and adolescence in the DPT-1, with a declining seroconversion rate with increasing age. Although, the risk of seroconversion for DAAs has been reported to be greatest in early life (<10 years) (10), risk of seroconversion exists throughout childhood and adolescence. While not in direct conflict with Colman et al. (4), the DPT-1 cohort provided additional information to possibly augment their recommendations to screen more than once before puberty.

GAD65 and ICA had the highest 2-year seroconversion risks, with GAD65 carrying a greater risk for those in early childhood (≤6 years of age). Seventy percent of those DAA positive in this cohort were identified at the initial screen, and rescreening efforts were successful at identifying an additional 10% of the expected 30% likely to seroconvert in the DAA-negative cohort. Of all the DAA-negative subjects eligible for rescreening ($n = 39,212$), the subjects that returned for rescreening ($n = 11,813$) were similar to the subjects that did not return for rescreening ($n = 27,399$) regarding age, sex, race, and relation to proband. By applying the absolute rate of seroconversion (469/11,813) to the entire negative cohort, an estimated 1,557 subjects would have likely seroconverted during the follow-up period. The initial screen identified 3,235 (70%) of the estimated 4,792 subjects likely to have seroconverted if the entire cohort was rescreened during the follow-up period. The estimated cost for a DAA screen (including all autoantibodies) is $70. Therefore, the initial screen cost per seroconversion was an estimated $918 ($70 for each of the 42,447 subjects at initial screen) to identify 70% of those who were DAA-positive versus $1,763 ($70 for each of the 11,813 subjects rescreened) for the remaining 10% ($n = 469$) that were identified through the DPT-1 rescreening effort.

Although economic realities may limit the ability to continually rescreen, strategies designed to capture those at highest risk are needed with the rapidly increasing incidence of type 1 diabetes

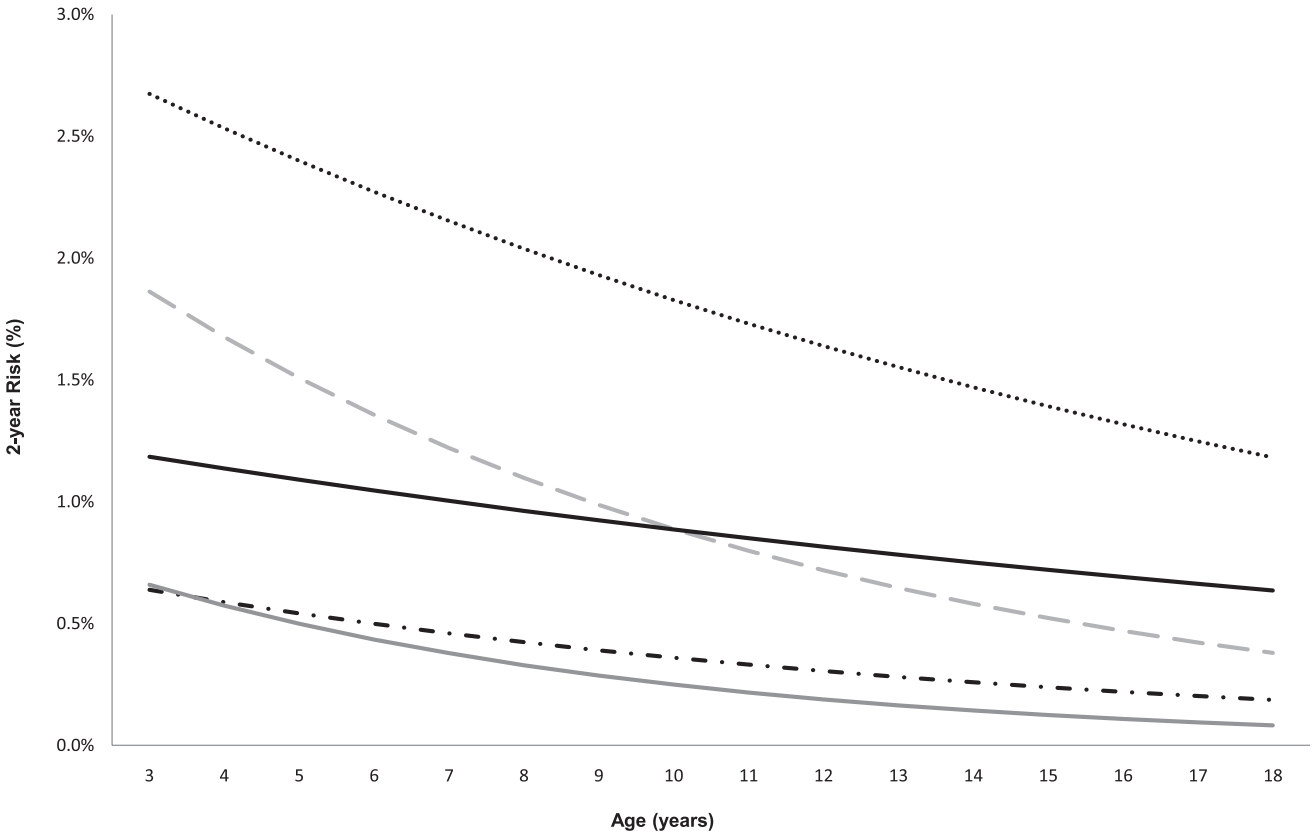

Figure 1—*Two-year risk of autoantibody seroconversion by autoantibody and development of any autoantibody(s) by age (years). GAD65, gray dashed line; ICA, solid black line; ICA512, black dashed line; any autoantibody, black dotted line; and any two autoantibodies, solid gray line.*

worldwide and the highest observed annual rates in the youngest age-group (5.4% [4.8–6.1] for children age 0–4 years) (11). The DPT-1 cohort data show that the majority of those that seroconverted were among the youngest and had the highest 2-year risk. As such, if we aim to identify the majority of subjects at risk for type 1 diabetes, it is necessary to continue screening for DAAs in early childhood, whereas screening in late adolescence and early adulthood will likely identify those with late-stage β-cell decline in whom therapy is not as effective. Based on these findings, annual rescreening for autoantibody seroconversion in relatives of type 1 diabetic probands is suggested for those in early childhood and adolescence and only once in late adolescence. This strategy is more cost effective to optimize identification of subjects at risk for DAAs and eligible for participation in prevention trials.

Despite the important and novel observations described, this study's main limitation was that timing of rescreening was not uniform across subjects. Subjects 3–10 years of age were rescreened annually, whereas subjects >10 years of age were rescreened biennially. Therefore, timing to seroconversion for those older than 10 years of age could occur anytime during a 2-year window. This difference in the screening interval for those >10 years of age could potentially underestimate the risk, where the time to DAA seroconversion could be a year earlier than observed. However, after assessment of the impact of this potential bias by adjusting for the screening cycle difference, age continued to be significantly associated with DAA seroconversion independent of the screening cycle. Also, it is likely the 2-year risks of DAA seroconversion may be underestimated because of the median age at initial screening in this cohort (~10 years); however, this provided additional support to initiate screening strategies earlier in life. Additionally, IAA seroconversion was not evaluated in this study because of limited measures on only a very small proportion of those rescreened. This limitation may be apparent in assessing risk for type 1 diabetes development, since IAAs have been shown to be a significant indicator of risk, as have ZnT8 autoantibodies. Lastly, this study was not able to assess risk for type 1 diabetes because of limitations in type 1 diabetes development follow-up on the screened population in the DPT-1.

Although many studies have assessed the presence of autoimmunity in type 1 diabetic high-risk subjects, the data have been limited in assessing screening strategies for type 1 diabetes autoimmunity. Understanding the etiology of the preclinical period of diabetes by determining the time of autoantibody seroconversion may assist in identifying potential environmental triggers associated with the etiology of islet autoimmunity.

This study provides evidence that the rate of autoantibody seroconversion declines with age, varies throughout childhood and adolescence, and extends into early adulthood. Those in early childhood have the highest 2-year risk for any DAA seroconversion (2.7% at 3 years), while the risk declines further from 1.8% at 10 years to 1.2% for those over 16 years of age. These findings suggest starting screening in early childhood for those at increased risk (i.e., those with a relative with type 1 diabetes) and screening annually for all DAAs (including ZnT8 autoantibodies and IAAs not measured

in this study) through early adolescence to capture the majority of those at risk for diabetes autoimmunity.

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