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Prediction of Autoantibody Positivity and Progression to Type 1 Diabetes: Diabetes Autoimmunity Study in the Young (DAISY)

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Abbreviations:

GAA
Antibodies to glutamic acid decarboxylase

HLA
     human leukocyte antigen
IA-2AA
     antibodies to protein tyrosine phosphatase IA2
IAA
     antibodies to insulin
ICA
     islet cell autoantibodies
PPV
     positive predictive value
T1DM
     type 1 diabetes mellitus

Diabetes Autoimmunity Study in the Young (DAISY) has followed 1972 children for islet autoimmunity and diabetes: 837 first-degree relatives of persons with type 1 diabetes and 1135 general population newborns identified through human leukocyte antigen (HLA) screening. During follow-up of 4.06 yr (range, 0.17–9 yr), serial determination of autoantibodies to glutamic acid decarboxylase, protein tyrosine phosphatase IA2, and insulin has generated approximately 20,000 results. Among 162 children with at least one positive autoantibody, in 31% the test was false positive (autoantibodies were negative twice on blinded duplicate aliquots), in 31% it was transiently positive (confirmed on blinded duplicate aliquots but negative on follow-up), and in 36% it was persistently positive.

Using proportional hazards modeling, the HLA-DR3/4 DQ8 genotype, another positive autoantibody at the first positive visit, and level of autoantibody were predictive of persistent positivity. Only HLA-DR3/4 DQ8 genotype was predictive of progression to diabetes in proportional hazards modeling. This prospective study reveals that cross-sectional determination of islet autoantibodies in a population with relatively low previous probability of autoimmunity identifies as "positive" a large number of individuals who are either false or transiently positive. Predictive value of autoantibodies increases with blinded duplicate and independent sample retesting and incorporation of the level of autoantibody in the predictive algorithm. (*J Clin Endocrinol Metab* 89: 3896–3902, 2004)

TYPE 1 DIABETES MELLITUS (T1DM) is characterized by autoimmune destruction of the pancreatic β-cells and marked by the production of antiislet autoantibodies. The first diabetes-associated autoantibodies described were islet cell autoantibodies (ICA) detected using sections of human pancreas. More recently, antibodies to insulin (IAA), glutamic acid decarboxylase (GAA), and the protein tyrosine phosphatase IA2 (IA-2AA) have been defined. Highly sensitive RIAs have been developed for the detection of IAA, GAA, and IA-2AA. Autoantibodies can be present years before the onset of diabetes, and progression to diabetes is associated with the presence of multiple autoantibodies that persist over time [1] [2] . These autoantibodies can also be expressed transiently. It is not known whether transient expression, which occurs at a greater frequency in people at a lower genetic risk of T1DM [3] , confers an increased risk of the disease.

The Diabetes Autoimmunity Study in the Young (DAISY) has followed, for the development of T1DM, a cohort of young first-degree relatives of type 1 diabetic patients and infants identified by newborn screening for human leukocyte antigen (HLA)-DR, DQ genotypes associated with diabetes. Through this prospective follow-up, children with antiislet autoantibodies have been discovered. Some of these children have progressed to diabetes, others remain antibody positive without yet developing T1DM, and still others have lost autoantibody expression. We have analyzed factors that are associated with confirmation of autoantibody positivity, the persistence of autoantibodies, and progression to diabetes.

Subjects and Methods

Subjects

DAISY is a prospective cohort study that has enrolled and followed, since 1993, 837 young first-degree relatives of patients with T1DM (sibling/offspring cohort) and 1,135 children identified through newborn screening for HLA-DR, DQ genotypes associated with T1DM (newborn cohort). Newborn cohort participants were identified by screening of umbilical cord blood obtained from over 27,000 newborns at St. Joseph's Hospital in Denver, Colorado. EDTA sample of cord blood was sent for HLA typing, as previously described [4] . The high-risk genotype (odds of T1DM by age 20 yr, 1:16) was defined as DRB1*04, DQB1*0302/DRB1*0301, DQB1*0201 (DR3/4 DQ8). Moderate risk genotypes (odds of T1DM by 20 yr, 1:75 in non-Hispanic whites and 1:230 in Hispanics) included DRB1*0301/*0301; DRB1*04, DQB1*0302/DRB1*04, DQB1*0302; and DRB1*04, DQB1*0302/x (where x is not DRB1*04, DQB1*0302, nor DRB1*0301 or DR2). The remainder of the genotypes were classified as average or low risk.

In the DAISY follow-up, all children are tested at 9, 15, and 24 months of age and annually thereafter for autoantibodies to pancreatic islet antigens (as described below). Children who are autoantibody positive are placed on an accelerated clinic visit schedule on which they return every 3–6 months for autoantibodies, hemoglobin A1c, and random blood glucose. Individuals who test negative for the autoantibodies on two consecutive visits return to the annual clinic visit schedule. All newborns with high risk and a subset with moderate or average risk HLA genotypes are invited to participate in the follow-up and have

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completed 4.5 (range, 1–15) visits on average. In addition, 170 general-population children identified at birth with the HLA-DRB1*0301, DQB1*0201/x genotypes (average risk for diabetes but high risk for celiac disease) were enrolled at the age of 2 yr and followed in identical manner with 2.5 (range, 1–8) visits, on average. Siblings and offspring of patients with T1DM are enrolled before age 4 yr, as soon as identified. The average age at the first visit in this group was 2.5 yr, and the average number of visits was 4.7 (range, 1–20). Of those, 105 of 837 (12.5%) relatives were first tested at 9 months of age and followed with 4.2 (range, 1–14) visits, on average.

The Combined Institutional Review Board at the University of Colorado Health Sciences Center has approved the DAISY project. Informed consent was obtained from the parents of each study subject at genetic screening (for the HLA-screened population) and again at enrollment in the DAISY follow-up.

Measurement of autoantibodies

GAA and IA-2AA were measured simultaneously by combined GAA and IA-2 radioassay (full-length glutamic acid decarboxylase and ICA512bdc cDNA clones). The assay was performed in the 96-well filtration plates with autoantibody-bound ^{3}H-glutamic acid decarboxylase and ^{35}S-IA-2AA precipitated with protein A-Sepharose. The cut-points for positivity were set at indexes of 0.032 (mean ± 2 sd, GAA) and 0.049 (mean ± 6 sd, ICA512AA), the 99th percentile of 198 normal controls. The interassay coefficient of variation of the assays is 6.5 and 9.6% for GAA and ICA512AA assays, respectively. Coefficients of variation are determined at high levels of positivity. In the Diabetes Autoantibody Standardization Program (DASP) 2002 proficiency workshop, assay specificity was 93% for GAA and 99% for ICA512AA, and sensitivity was 90% for GAA and 62% for ICA512AA.

As previously described [5] , the micro-IAA assay in this study used ^{125}I-insulin in standard 96-well filtration plates that quantitate precipitated ^{125}I-insulin with a multichannel 96-well β-counter. Scintillation fluid is directly added to the 96-well plate (Top Counter scintillation counter; Hewlett-Packard, Palo Alto, CA). The upper limit of normal (0.01) was chosen as the 99th percentile from receiver operator curves in 106 healthy control subjects and 105 patients with new-onset diabetes. In the 2002 DASP workshop, sensitivity was 62% and specificity was 98%. The intra- and interassay coefficient of variation is 12 and 16%, respectively. Coefficients of variation are determined at high levels of positivity. Indices for all
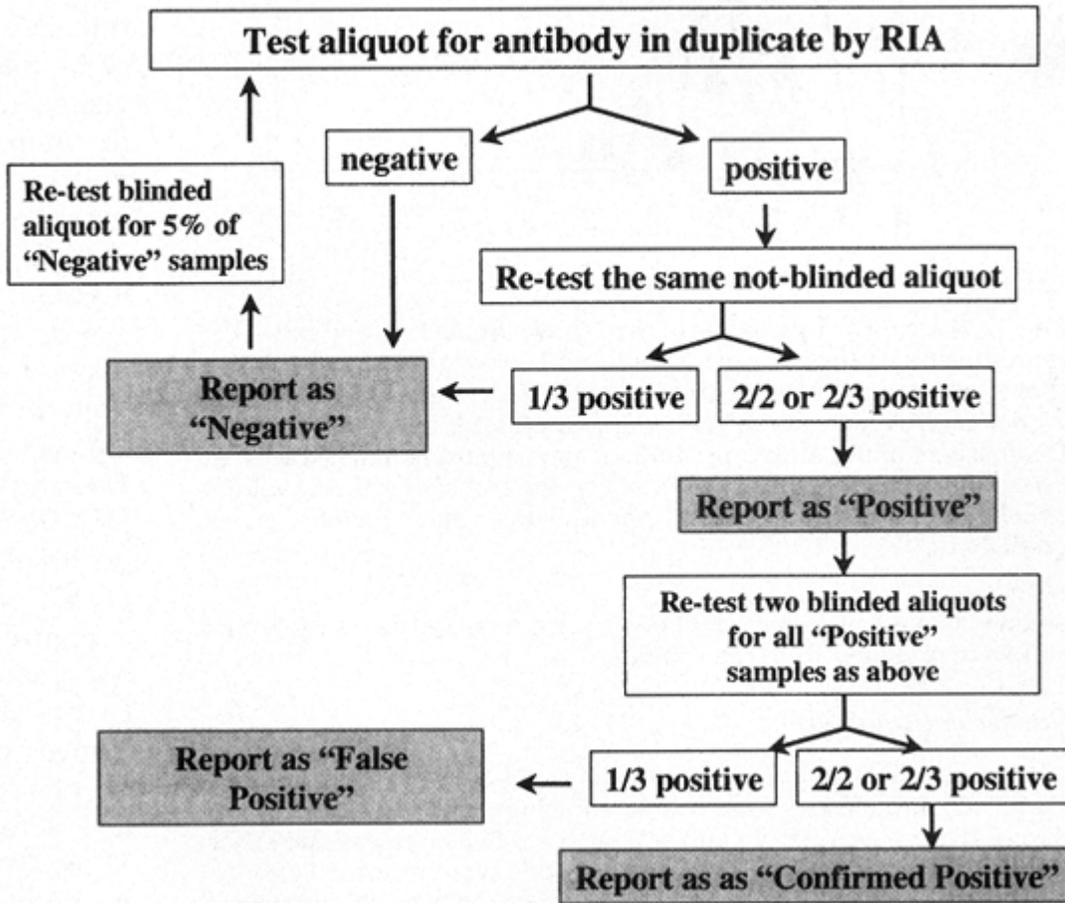
Figure 1. Flow diagram for classification of positive and negative status in the DAISY study.

autoantibodies were calculated using a consensus value for negative and positive control specific for that autoantibody.

DAISY uses an extensive system of quality assurance to correctly classify autoantibodies as positive or negative (Fig. 1). The autoantibodies are measured by RIA in duplicate. Autoantibodies are classified as positive when average level of the duplicate is greater than the 99th percentile of the normal population. If the initial measurement is negative, the sample is reported as negative; however, a random 5% of these samples is retested in a blinded fashion. If the initial result is positive, the assay is repeated in unblinded fashion on the same aliquot and if two of two or two of three repeat tests are positive, the result is reported as positive. However, if only the initial one of three repeat tests is positive, it is reported as negative. When DAISY staff, independent of the laboratory, receive a positive test report, two additional blinded aliquots of the original sample are sent to the laboratory, and the process described above is repeated independently for both aliquots. The blinded aliquots are repeated in separate assays. To be confirmed as positive, the sample has to be reported as positive on at least two of three blinded aliquots (and with two of two or two of three tests positive for each aliquot). If the autoantibody fails to confirm (only the original aliquot is positive), the final result is classified as a false positive. Therefore, a confirmed positive result is based on at least four positive tests.

Individuals included in this analysis were those that had at least one positive autoantibody test, including an unconfirmed one. False-positive results were defined as above, with a positive autoantibody test not confirmed on quality-control aliquots. Persistent autoantibodies were defined as confirmed positive for at least one autoantibody at more than one visit, including the last visit. This group was further divided into those who have progressed to T1DM, prediabetic, and those who have remained diabetes-free, persistent nondiabetic. If a child was previously confirmed positive, but negative at the last sample, the autoantibody was defined as transient and further divided into those with a single positive result or those with multiple positive samples over time before becoming negative. Figure 2 illustrates autoantibody patterns of selected individuals in the transiently positive, persistent nondiabetic, and prediabetic groups. These groups were compared for HLA genotypes, family history of T1DM, level of autoantibody positivity, and number of positive autoantibodies.

Individuals whose antibody measurements were positive at less than 12 months of age and became negative on the next sample or whose cord
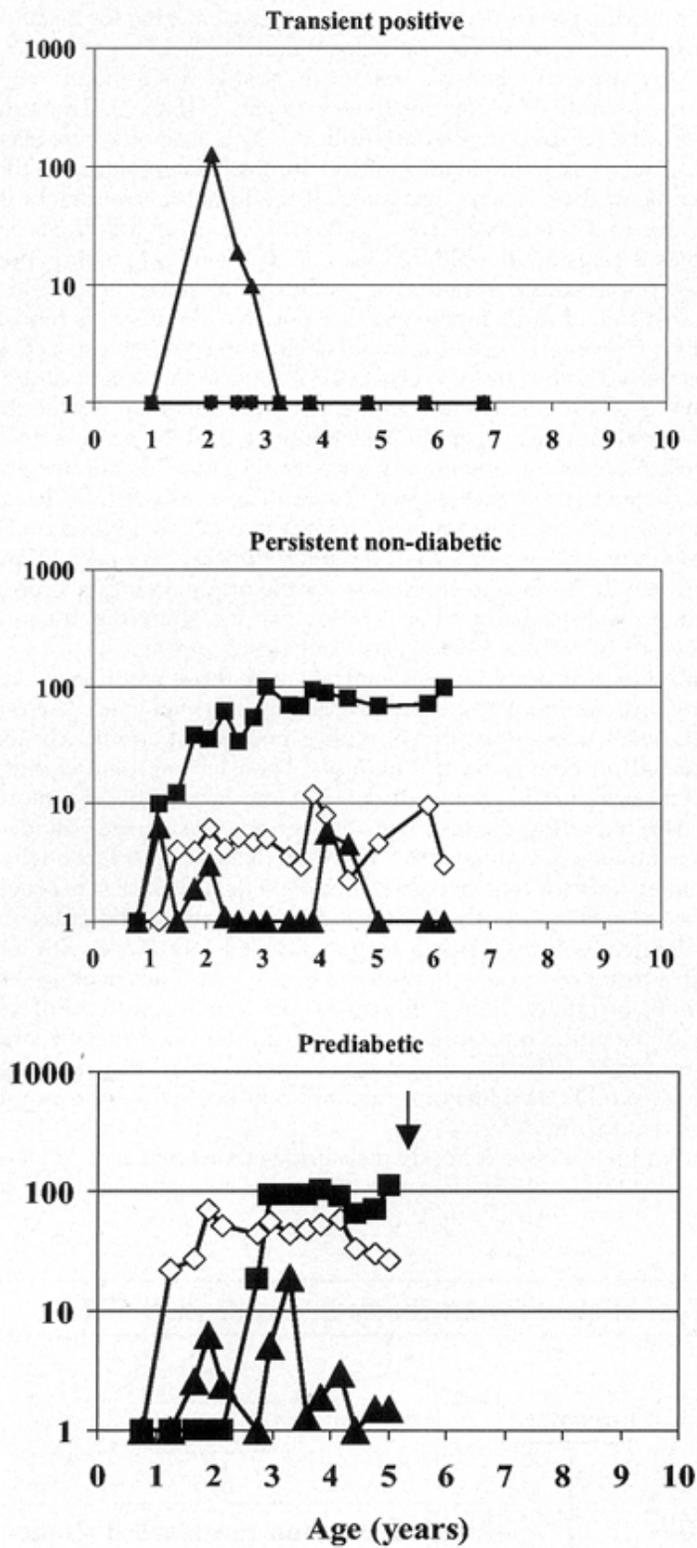
Figure 2. Autoantibody profiles of three individuals followed in DAISY representing transient, persistent, and prediabetics. The y-axis shows SD score for autoantibody level. GAA is shown as an *open diamond*, IAA as a *closed triangle*, and IA-2AA as a *closed square*. Diagnosis of diabetes in a prediabetic participant is marked with an *arrow*. *Top panel*, Transiently positive for IAA three times; *middle panel*, persistently positive nondiabetic; and *bottom panel*, prediabetic.

blood was positive for autoantibodies were classified as transplacental and were excluded from the analysis.

Statistical analysis

Statistical analyses were performed using SAS 8.0 (SAS Institute, Cary, NC). Proportions were compared using the χ^2 test unless 20% of the cells had an expected value less than five, in which case Fisher's exact test used. Analysis for levels of autoantibody was performed after log transformation to normalize the distribution. ANOVA with Bonferroni correction to control for multiple comparisons was used to compare transformed autoantibody levels in various study subgroups.

Survival analysis was performed using proportional hazards modeling with progression to diabetes or development of persistent positivity as the outcome variable in the model included positive family history of T1DM, HLA genotype, number of autoantibodies positive, and the highest level of first positive autoantibody. In the survival model, time to diabetes was calculated as the difference between diabetes development and first positive autoantibody. Time to persistent positivity was defined as the difference between last visit at which the individual was still positive and first positive autoantibody. The highest level of first positive autoantibody was determined for each individual at the visit in which the individual was positive for the first time for at least one autoantibody. When more than one autoantibody was positive at the same visit, the autoantibody with the highest level was chosen. Analysis for levels of autoantibody was performed after log transformation to normalize the distribution. Individuals with confirmed positive autoantibodies were included in the proportional hazards modeling.
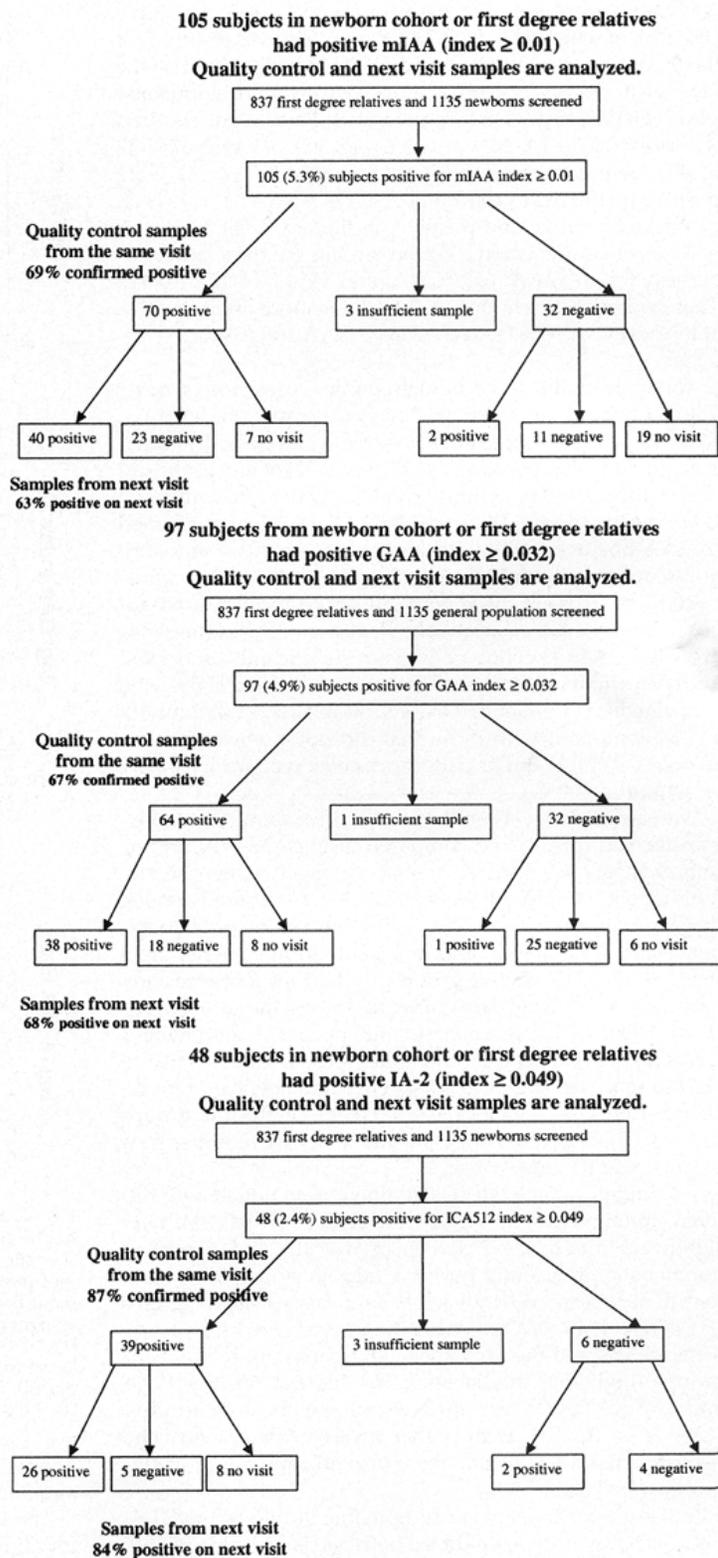
Positive predictive values (PPVs) were calculated. Individuals included in this analysis were followed from 1.25 yr or less to ensure that they were identified as close to the initiation of autoantibody positivity. Levels were defined as positive at greater than or equal to 2, 5, or 10 sD above the mean. If multiple levels were positive at the first visit, the highest level was used.

Results

During the follow-up, 162 of 1972 (8.2%) children had at least one positive autoantibody, including those positive for one, two, or three different autoantibodies and those that were false, transiently, or persistently positive. Thirty-one percent (50 of 162) of the children were false positive, and none has developed T1DM. The remaining 69% (112 of 162) were confirmed positive. Rates of false-positive results for the individual autoantibodies have remained stable over the 9 yr of the study (~0.5% of GAA, 1.1% of IAA, and 0.1% of IA-2AA). Of those confirmed positive, in 50 children the autoantibodies were transient, in 58 persistently positive, and in four children the autoantibodies were positive once at the last or only visit and therefore could not be classified as transient or persistent. In the transiently positive group, 22 of 50 children were positive at more than one visit (mean, 3.3 visits), but only 2 of the 50 transiently positives were positive for more than one autoantibody. None of the transiently positive children has developed diabetes. In contrast, in the persistently positive group, 24 of 58 children have progressed to diabetes and 34 continue to be persistently positive but nondiabetic.

The proportion of individuals with a first-degree T1DM relative was lower: 34% (17 of 50) in the false-positive children compared with 60% (30 of 50) in transiently positive and 76% (44 of 58) of the persistently positive group ($P < 0.0001$). The proportion of children with the highest risk HLA-DR3/4 DQ8 genotype differed in similar manner, with 20% of false positive, 20% of transiently positive, and 48% of persistently positive children carrying this genotype ($P = 0.001$).

Figure 3, A–C , shows the flow diagram for the autoantibody assays. One hundred five children were found to be IAA positive (Fig. 3A). Of these, 70 of 102 (69%) were confirmed positive on analysis of blinded aliquots (three samples had insufficient volume). Of children confirmed positive, 40 of 63 (63%) were positive on their subsequent visit. Thus, 43% (69% × 63%) were both confirmed positive and positive on the next visit.

105 subjects in newborn cohort or first degree relatives
had positive mIAA (index ≥ 0.01)
Quality control and next visit samples are analyzed.

| 837 first degree relatives and 1135 newborns screened |

| 105 (5.3%) subjects positive for mIAA index ≥ 0.01 |

Quality control samples
from the same visit
69% confirmed positive

| 70 positive | | 3 insufficient sample | | 32 negative |

| 40 positive | 23 negative | 7 no visit | | 2 positive | 11 negative | 19 no visit |

Samples from next visit
63% positive on next visit

97 subjects from newborn cohort or first degree relatives
had positive GAA (index ≥ 0.032)
Quality control and next visit samples are analyzed.

| 837 first degree relatives and 1135 general population screened |

| 97 (4.9%) subjects positive for GAA index ≥ 0.032 |

Quality control samples
from the same visit
67% confirmed positive

| 64 positive | | 1 insufficient sample | | 32 negative |

| 38 positive | 18 negative | 8 no visit | | 1 positive | 25 negative | 6 no visit |

Samples from next visit
68% positive on next visit

48 subjects in newborn cohort or first degree relatives
had positive IA-2 (index ≥ 0.049)
Quality control and next visit samples are analyzed.

| 837 first degree relatives and 1135 newborns screened |

| 48 (2.4%) subjects positive for ICA512 index ≥ 0.049 |

Quality control samples
from the same visit
87% confirmed positive

| 39 positive | | 3 insufficient sample | | 6 negative |

| 26 positive | 5 negative | 8 no visit | | 2 positive | 4 negative |

Samples from next visit
84% positive on next visit

Figure 3. Flow diagram for positive autoantibody assay and quality-control and follow-up samples. *Top*, The flow diagram for all individuals with an initial assay positive for IAA. The initial positive is confirmed 69% of the time and the follow-up sample positive 63% of the time. *Middle*, The flow diagram for all individuals with an initial assay positive for GAA. The initial positive is confirmed 67% of the time and the follow-up sample positive 68% of the time. *Bottom*, The flow diagram for all individuals with an initial assay positive for IA-2AA. The initial positive is confirmed 87% of the time, and the follow-up sample is positive 84% of the time.

Ninety-seven children were found to be GAA positive (Fig. 3B), of which 64 of 96 (67%) were confirmed positive. Of those confirmed positive, 38 of 56 (68%) were positive on the next visit. Thus, 46% (67% × 68%) were both confirmed positive and positive on the next visit. Forty-eight children were found to be IA-2AA positive (Fig. 3C), of which 87% (39 of 45) were confirmed positive and 84% (26 of 31) were positive on the next visit. Thus, 73% (87% × 84%) were both confirmed positive and positive on the next visit.

Rates of confirmation differed among the three autoantibodies: 87% IA-2AA, 67% GAA, and 69% IAA ($P = 0.04$). There was also a trend for IA-2AA to be more often positive at the next visit: 84% IA-2AA *vs.* 68% GAA and 63% IAA ($P = 0.13$).

For each of the three autoantibodies, presence of other autoantibodies was associated with confirmation. For IAA, 54% (38 of 70) of confirmed positive children had another autoantibody compared with 19% (six of 32) of those who did not confirm ($P = 0.001$). Similarly, 42% (27 of 64) of confirmed GAA-positive individuals and 85% (33 of 39) of confirmed IA-2AA-positive individuals had another positive autoantibody compared with 19% (six of 32) ($P = 0.04$) and 50% (three of six) ($P = 0.08$) of those who did not confirm positive for GAA and IA-2AA, respectively. Presence of the highest-risk HLA-DR3/4 DQ8 genotype was not significantly associated with confirmation for any of the autoantibodies. Those who confirmed for GAA tended to have more often a relative with T1DM compared with those that did not confirm (70% *vs.* 50%; $P = 0.07$); a similar pattern was observed for IAA (65% *vs.* 44%; $P = 0.05$).

Autoantibody levels on the first positive sample were log-transformed (Fig. 4) and compared, using ANOVA, among four groups: false positive, transiently positive, persistently nondiabetic, and prediabetic. IAA levels differed among these four groups ($P < 0.0001$), with those in the prediabetic, persistent nondiabetic, and transient groups higher than those in the false-positive group ($P < 0.05$ for pairwise comparisons). GAA levels also differed among the four groups ($P < 0.0001$) with prediabetic and persistent nondiabetic levels higher than transiently or false positive ($P < 0.05$) and no difference between the persistent nondiabetic and prediabetic groups. IA2-AA levels differed among the four groups ($P = 0.03$), whereas none of the pairwise comparisons reached significance.

The highest positive levels attained (data not shown) differed among the four groups for IAA ($P < 0.0001$), with significant differences between prediabetic and persistent nondiabetic, transiently positive, false-positive, and persistent nondiabetic and transiently and false-positive groups ($P < 0.05$). IAA was the only autoantibody in which levels differed between the prediabetic and persistent nondiabetic groups (higher in prediabetic). The highest positive GAA and IA2-AA levels also differed among the four groups' GAA ($P < 0.0001$), with higher levels in prediabetic and persistent nondiabetic than the transiently and false-positive groups ($P < 0.05$).

In the proportional hazards modeling (Table 1), the HLADR3/4 DQ8 genotype predicted both persistence of autoantibodies and progression to diabetes. The level of the autoantibody

First positive IAA

First positive GAA

First positive IA-2AA
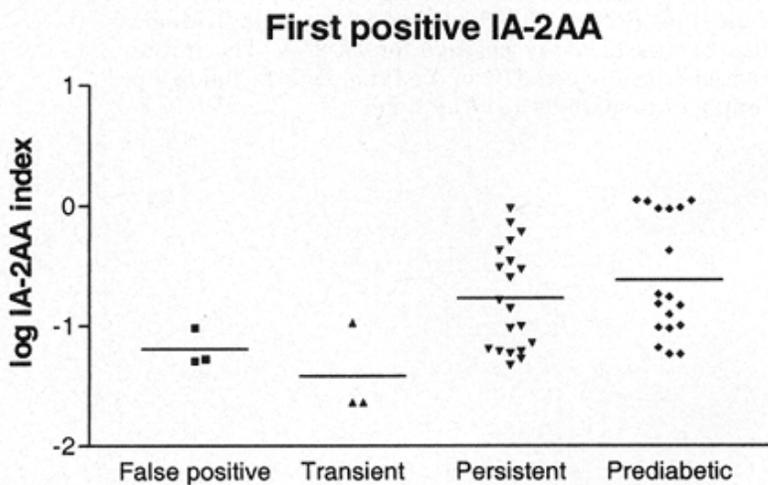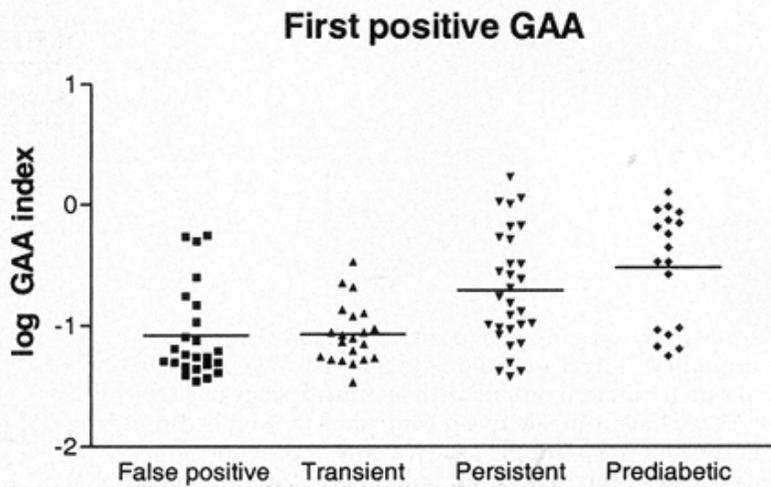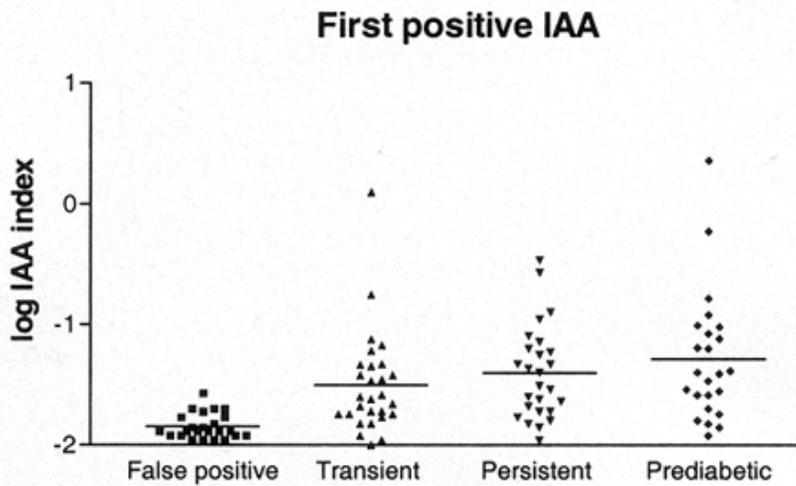
Figure 4. Level of first positive autoantibody. The level of autoantibody has been transformed to log scale for normalization. The x-axis shows the four different groups: false positive, transiently positive, persistent positive, and prediabetic. Only individuals positive for the autoantibody are shown. After controlling for multiple comparisons, IAA is higher in persistent and prediabetics compared with false positive ($P < 0.0001$ for the model). GAA is higher in persistent and prediabetics compared with false and transient positives ($P < 0.0001$ for the model). IA-2AA shows significant difference across the four groups ($P = 0.03$), without any pair showing a difference.

at the initial sample and presence of another autoantibody predicted persistence.

The PPV for persistent positive was 41.6, 69.1, and 82.4% for autoantibody levels greater than or equal to 2, 5, and 10 SD above the mean. The PPV for diabetes was 14.2, 20.0, and

TABLE 1 -- Proportional hazards modeling

Variable	Hazard ratio (95% CI)	P value
Persistent positivity in confirmed autoantibody-positive group		
HLA DR3/4 DQ8 (n = 37, 28 persistent positive)	1.99 (1.14–3.47)	0.019
First-degree relative (n = 74, 44 persistent positive)	1.05 (0.56–1.96)	0.9
Another positive antibody at first positive visit (n = 27, 25 persistently positive)	2.55 (1.27–5.11)	0.008
Level of the first positive autoantibody	1.71 (1.02–2.86)	0.048
Progression to diabetes in confirmed autoantibody positive group		
HLA DR3/4 DQ8 (n = 37, 14 with T1DM)	4.32 (1.78–10.47)	0.01
First-degree relative (n = 74, 21 with T1DM)	1.63 (0.58–4.55)	0.38
Another positive antibody at first positive visit (n = 27, 14 with T1DM)	2.01 (0.80–5.07)	0.15
Level of the first positive autoantibody	1.10 (0.52–2.34)	0.80

Proportional hazards modeling was performed on those with confirmed positive autoantibodies. The variables HLA DR 3/4 DQ 8, first-degree relative with T1DM, another antibody positive at the first positive visit, and level of first positive autoantibody were included in the model. Listed are the total number of individuals with each variable (n) and the number who are either persistently positive or diabetic. CI, Confidence interval.

26.5% for autoantibody levels greater than or equal to 2, 5, and 10 SD above the mean, respectively.

Discussion

Prediction of T1DM is an area of intense investigation. Factors such as family history and HLA genotypes are known to increase the risk of T1DM. Despite the fact that the majority of individuals with T1DM do not have a family history of disease, those who do have a family history have an increased risk over the general population (6% compared with 0.4%) [6] . Twin studies also confirm strong genetic contribution to T1DM risk [7] [8] . In our study, the presence of a family history of T1DM was found to be associated with confirmation of autoantibody positivity. However, when analyzed in proportional hazards modeling, it was no longer an independent predictor of persistent positivity or diabetes in a group of individuals with positive autoantibodies. Approximately 50% of the genetic risk for T1DM is attributable to the HLA region [9] . In proportional hazards modeling, the presence of the high-risk HLA-DQ3/4 DQ8 genotype was associated with persistence and progression to diabetes in a group of confirmed positive individuals.

The expression of ICA and the biochemical autoantibodies GAA, IAA, and IA-2AA identify individuals at a high risk of T1DM. These autoantibodies can be detected during infancy [10] [11] and usually precede T1DM by many years [12] [13] . Characteristics of the autoantibody expression have been used to predict T1DM. The persistent production of autoantibodies has been associated with T1DM. In the Finnish study, the Type 1 Diabetes Prediction and Prevention (DIPP), which identifies children at risk for T1DM with HLA screening [14] , 15 children progressed to T1DM. None had reverted to negative autoantibodies before diabetes, and 14 of the 15 expressed IAA, which was usually the first autoantibody to develop [11] . In our group, all children who developed diabetes were persistently positive for at least one autoantibody at the time of diagnosis with 23 of 24 positive for more than one autoantibody. Twenty-two of 24 were positive for IAA, 18 were positive for GAA, and 18 for IA-2AA. The German BABY-DIAB study [15] [16] has followed children of parents with T1DM for the development of islet autoimmunity and T1DM. Those children who went on to diabetes were positive for both ICA and IAA.

ICA levels have been associated with progression to diabetes [17] [18] . Levels of ICA greater than 20 Juvenile Diabetes Foundation units were associated with a 53% 10-yr risk of diabetes, compared with a 43% risk for patients with levels greater than 10 Juvenile Diabetes Foundation units [1] . Progression to diabetes has previously been associated with the number of biochemical autoantibodies and their levels [1] [2] . A population-based study in school children found that higher autoantibody levels were associated with a higher specificity for diabetes, at the expense of sensitivity [2] . Linking the levels of ICA and biochemical islet autoantibodies, levels of ICA were associated with the expression of multiple biochemical islet autoantibodies [19] . Adding to the complexity of diabetes prediction, HLA has been associated with autoantibody level. HLA DR4 has previously been associated with insulin autoantibody level in the competitive insulin autoantibody assay [20] . In our study, higher levels of each of the autoantibodies were associated with confirmation, persistence of autoantibodies, and development of T1DM. In particular, higher peak levels of IAA were associated with a greater progression to T1DM. In the proportional hazards model, the level of autoantibody was only predictive of persistent positivity, not reaching statistical significance to predict T1DM.

Autoantibody assays are problematic, especially when positive values are defined as above the 99th percentile in low previous probability populations (as in this study). This explains the high proportion of individuals with false-positive autoantibodies in this study (31%). Extensive checks and balances need to be in place to ensure the most accurate assessment of autoantibody level. In particular, before the first serum aliquot in DAISY is reported as positive, it is confirmed in separate assays with two of three positive tests defining positivity.

A group of particular interest is those that transiently express autoantibodies. This group has been identified in other studies, such as DIPP in which almost half of auto-antibody-positive children reverted to negative [14] , similar to our study in which 45% of confirmed autoantibodies were

transiently positive. This group remains a mystery. It is possible that transient expression is due to assay variability. However, when considering the confirmatory process performed in the DAISY study and the fact that 22 of 50 individuals with transient autoantibodies were positive on multiple occasions (with at least four separate assays higher than the 99th percentile to be confirmed on any single visit), it is unlikely that assay variability alone accounts for transient positivity. In contrast, transient expression may represent "transient" islet cell autoimmunity that was somehow reversed. Identifying those that can reverse the autoimmune process may identify factors that may be critical to the development of diabetes. Transient expression of autoantibodies in childhood may be associated with the return of positive antibodies in later childhood and adulthood, or development of diabetes as an adult due primarily to β-cell defects. Only with continued follow-up will the natural history of transiently positive individuals be clarified.

Of interest, in our group one individual who progressed to T1DM was positive for IAA and then negative at two visits and then returned to positivity before developing diabetes. Two individuals initially persistently positive were positive for IA-2AA, became negative, and then developed GAA in one case and GAA, IA-2AA, and IAA in the other. They still have not progressed to diabetes.

Although our study has been enrolling patients since 1993, allowing for a relatively long follow-up period, the time course of progression from autoimmunity to T1DM is over many years and maybe even decades. Therefore, many of the individuals identified as persistent positives in this study who have not yet developed diabetes may still go on to develop diabetes. The distinction between the persistent positive and diabetic group therefore may not be a qualitative one. The follow-up time of individuals who have developed diabetes is shorter than those who have remained persistently positive and not yet diabetic (3.49 *vs.* 5.49 yr; $P = 0.002$). Those who have progressed to clinical diabetes likely experience a more aggressive autoimmune attack of the pancreas.

Taken in aggregate, the use of the level of autoantibody can provide additional predictive information for the persistence of autoantibodies and development of T1DM and may be used to further stratify individuals for epidemiological and preventive studies.

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