

# Interaction of Onset and Duration of Diabetes on the Percent of GAD and IA-2 Antibody-Positive Subjects in the Type 1 Diabetes Genetics Consortium Database

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**OBJECTIVE**—GAD antibodies (GADA) are more common in type 1 diabetic subjects diagnosed at an older age, whereas insulinoma-antigen 2 antibodies (IA-2A) are more common in subjects with younger onset. The prevalence of both antibodies decreases with longer duration of type 1 diabetes. We evaluated the interaction between age of diagnosis (onset) and duration of diabetes on the percentage of GADA- and IA-2A-positive subjects.

**RESEARCH DESIGN AND METHODS**—Data were used from 5,020 individuals with type 1 diabetes obtained from the Type 1 Diabetes Genetics Consortium dataset. The percentages of GADA- and IA-2A-positive subjects were modeled with duration as the continuous independent variable using a modified spline.

**RESULTS**—Within the first 5 years from diagnosis, 19.4% of individuals (median age 13 years) had neither GADA nor IA-2A, and by 6 to 13 years after diagnosis (median age 18 years), 31.7% were antibody-negative. There was no significant interaction between onset of disease and duration of diabetes for IA-2A ( $P = 0.30$ ). The interaction was significant for GADA ( $P = 0.0002$ ), resulting from differences in subjects diagnosed at or older than age 14. For these individuals, there was no apparent effect of duration of disease on the percentage of GADA-positive subjects within the first 5 years of diagnosis.

**CONCLUSIONS**—Onset and duration of diabetes both have an important effect on antibody status. The interaction of onset and duration on GADA positivity, but not on IA-2A, suggests differences in biology. These data provide a context for clinicians to interpret results of autoantibody testing in clinical practice.

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**D** iabetes autoantibodies (DAAs) have been used to predict risk for type 1 diabetes and to classify individuals with diabetes as having an immune-mediated  $\beta$ -cell destructive process. At diagnosis of type 1a diabetes, about 95% of individuals will have one or more autoantibodies, including insulin autoantibodies (IAA), GAD antibodies (GADA), insulinoma-antigen 2 antibodies (IA-2A,

also called ICA512), and the recently described zinc transporter protein autoantibodies (ZnT8Ab) (1). The frequency of antibody positivity is known to vary with age and to decrease with longer duration of disease. For example, GADA are more common in older subjects, whereas IAA and IA-2A are more common in younger individuals (2–6). About 45% of subjects are positive for GADA or

IA-2A about 15 years from diagnosis (2). HLA type is also associated with antibody frequency, with GADA more common in DR3 (7,8) individuals with type 1a diabetes and IA-2A more common in DR4 individuals (7–10).

What, if any, interaction there is between age of diagnosis and duration of diabetes on GADA and IA-2A status is unknown. We explored this question using the large Type 1 Diabetes Genetics Consortium (T1DGC) dataset of individuals with type 1 diabetes who provided blood samples for genetic analysis and autoantibody typing. Our primary objective was to investigate the interaction of age of diagnosis (onset) and duration of diabetes on GADA and IA-2A status in subjects from the T1DGC.

## RESEARCH DESIGN AND METHODS

### Subjects

Data were obtained from a July 2009 download of the cross-sectional T1DGC database. This international consortium was designed to collect data and samples from families with type 1 diabetes to investigate the contribution of genetics, including HLA type, in the development of type 1 diabetes (11). Samples and data were obtained at multiple institutions after appropriate human subjects review and written consent. Samples were tested for GADA and IA-2A at the Barbara Davis Center (Denver, CO) using previously described assays (12).

Two groups of families participated: affected sib-pair (ASP) families, defined as families in which at least two non-monozygotic siblings had type 1 diabetes, and families in which there was a single affected child from a population with a low prevalence of type 1 diabetes (trios). Affected siblings were considered eligible for T1DGC if they were diagnosed with type 1 diabetes before age 35 and treated with insulin within 6 months of diagnosis

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without subsequent discontinuation of insulin treatment. After review by the eligibility committee, 25 siblings with onset after age 35 were also included.

**Analysis**

Associations of antibody positivity with age at onset, duration of diabetes, and HLA typing were estimated using logistic regression models. Separate models were fit for GADA, IA-2A, and the occurrence of either. Generalized estimating equations were used in all regression models to account for potential correlation between siblings. Onset and duration were categorized by tertiles. Multivariate models were fit, including the categorical onset and duration variables and interactions, along with HLA typing. A model was also fit that modeled prevalence of the outcome as a function of the categorical onset variable and a spline function for duration. The spline was a continuous function that was linear within each tertile of duration, but the slope of which could vary between tertiles. The Wald test was used to calculate the significance of the terms within each model. A nonparametric scatter plot smoother program (13) was used to assess model fit. Analyses were computed using R 2.11.0 software (14). Values of  $P < 0.05$  were considered statistically significant.

**RESULTS**—Complete HLA typing and antibody data were available for 5,315 subjects. However, more than 40% of children with duration of  $\leq 3$  years and

who were diagnosed with diabetes  $< 2$  years of age were negative for both GADA and IA-2A (Supplementary Fig. 1). Because this subpopulation may include patients with monogenic diabetes, we excluded from all subsequent analysis 295 individuals who were aged  $< 2$  years when they were diagnosed with type 1 diabetes. Data for the remaining 5,020 subjects were used in all subsequent analysis.

The median age at diagnosis was 10 years (range 2–52). The median duration of disease at the time of blood sampling was 8 years (range 0–66). A total of 4,817 subjects were from ASP families, including 157 parents, and 203 subjects with type 1 diabetes were from trio families, of which eight were parents. Males comprised 50.7% of subjects, 4,739 individuals were white/Caucasian, 191 (3.8%) were Asian, 80 (1.6%) were black/African American, and 13 (0.3%) were Pacific Islanders. Separate univariate and multivariate analysis examining race are available in the Supplementary Tables 1 and 2, respectively.

**Univariate analysis**

GADA were more common in subjects who were older at the time of diagnosis, with an odds ratio (OR) of 2.57 (95% CI 2.21–3.00) in those aged  $> 13$  years when they were diagnosed compared with the reference group of those aged  $< 8$  years at diagnosis (Table 1, Fig. 1A). For IA-2A, subjects who were diagnosed in the

middle tertile (8–13 years) had significantly higher rates of IA-2A positivity than those with earlier (2–7 years) or later onset ( $\geq 14$  years; Table 1, Fig. 1B). Subjects with increasing duration of type 1 diabetes were less likely to be GADA- or IA-2A-positive (Table 1, Fig. 1C and D). GADA were positively associated with DR3 and DR4, with the exception of DR4/X (where X is not DR3 or DR4), which was not significant. IA-2A was also positively associated with DR4 in any combination, but was negatively associated with DR3/DR3 (Table 1).

GADA and/or IA-2A positivity (subsequently denoted  $Aby^+$ ) was an admixture of the results for GADA and IA-2A (Table 1). Specifically, subjects with onset of diabetes at age  $\geq 8$  years were more likely  $Aby^+$  than those diagnosed when they were younger.  $Aby^+$  was less likely with increasing duration of diabetes. DR3 and DR4 were both positively associated with  $Aby^+$ .

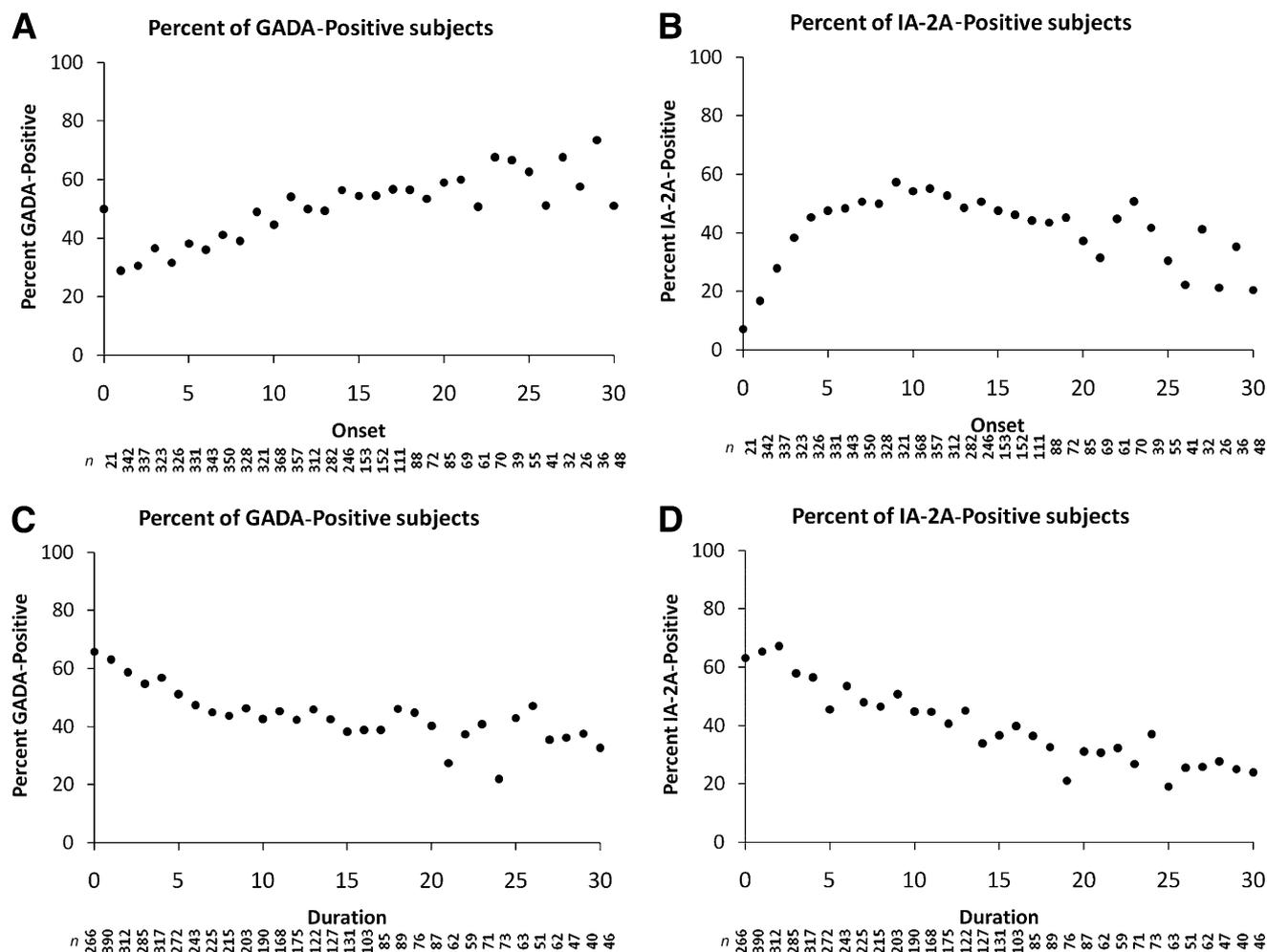
**Multivariate analysis**

Our initial multivariate models for GADA, IA-2A, and  $Aby^+$  included only age of onset and duration of disease as independent variables (Supplementary Table 3). Adding HLA to each of these models (Table 2) improved the fit ( $P < 0.0001$  for each HLA-adjusted model compared with non-HLA-adjusted model), yet had very little impact on the OR from the non-HLA-adjusted model (Supplementary Table 3). In contrast to the univariate analysis, subjects with onset of 8 to 13 years

**Table 1—Univariate analysis for GADA, IA-2A, or GADA and/or IA-2A subjects\***

Covariate	N	GADA			IA-2A			$Aby^+$		
		% Pos	OR	95% CI	% Pos	OR	95% CI	% Pos	OR	95% CI
Onset age (years)										
2–7	1,739	35.7	1	—	43.1	1	—	60.3	1	—
8–13	1,767	47.6	1.64†	1.42–1.88	53.1	1.50†	1.31–1.72	71.6	1.67†	1.44–1.92
$\geq 14$	1,514	58.9	2.57†	2.21–3.00	40.6	0.90	0.78–1.04	69.4	1.50†	1.28–1.74
Duration (years)										
0–5	1,842	58.6	1	—	60.4	1	—	80.6	1	—
6–13	1,541	44.8	0.57†	0.50–0.66	47.2	0.58†	0.51–0.67	68.3	0.52†	0.44–0.61
$\geq 14$	1,637	35.6	0.39†	0.34–0.45	28.3	0.26†	0.22–0.30	50.6	0.25†	0.21–0.29
HLA										
X/X	374	36.6	1	—	30.2	1	—	50.0	1	—
DR3/X	627	49.6	1.70‡	1.27–2.27	26.0	0.81	0.60–1.10	57.3	1.34§	1.01–1.78
DR3/DR3	374	57.0	2.29†	1.66–3.16	22.7	0.68§	0.47–0.98	65.2	1.88‡	1.36–2.59
DR3/DR4	1,847	47.9	1.59§	1.23–2.05	49.1	2.23†	1.72–2.89	70.2	2.35†	1.83–3.02
DR4/X	1,278	41.8	1.24	0.96–1.61	56.7	3.02†	2.31–3.95	69.0	2.23†	1.72–2.89
DR4/DR4	520	52.5	1.91†	1.41–2.59	59.8	3.44†	2.52–4.68	76.3	3.23†	2.37–4.40

\*Individual univariate analyses; dependent variables are GADA, IA-2A, or  $Aby^+$ , and independent variables (covariate) are either age of onset, duration of disease, or HLA type.  $Aby^+$  indicates those who are GADA- and/or IA-2A-positive. % Pos, percentage of antibody-positive subjects; X  $\neq$  DR3 or DR4. † $P < 0.0001$ ; ‡ $P < 0.001$ ; § $P < 0.05$ .



**Figure 1**—The percentage of antibody-positive subjects is affected by the onset and duration of type 1 diabetes for GADA (A and C, respectively) and IA-2A (B and D, respectively). Onset was limited to age  $\geq 2$  years for panels C and D, as described in the text. Given an increase in the scatter (due to lower numbers of subjects), the x-axis is truncated at both an onset and duration of 30 years. n, number of subjects.

were statistically no more likely to be IA-2A-positive than subjects with onset of 2 to 7 years when duration was limited to  $\leq 5$  years (Table 2).

#### Interaction of onset and duration on antibody positivity

The smoothed plots for GADA, IA-2A, and  $\text{Aby}^+$  are shown in Fig. 2. Figure 2A suggests a possible interaction of onset and duration for GADA, whereas this is not evident for IA-2A or having at least one antibody present ( $\text{Aby}^+$ ). This was confirmed using a modified spline in which a significant interaction of onset and duration was found for GADA ( $P = 0.0002$ ), but not for IA-2A or  $\text{Aby}^+$  (Fig. 3).

The interaction for GADA was driven by differences in the frequency of GADA positivity among those who were age 14 or older when diagnosed compared with subjects who were younger at the time of

diagnosis. Specifically, for these subjects, there was no apparent effect of duration of disease on the percentage of GADA-positive subjects within the first 5 years of diagnosis. Over the next 8 years of duration of disease, the decline in the percentage of GADA-positive subjects approximately parallels that of the other tertiles of onset. The percentage of GADA-positive subjects declines more rapidly in those who were diagnosed age 14 or older compared with those who were younger at the time of diagnosis. Removing the 365 outliers with a diabetes duration of  $>30$  years from our model resulted in similar results (Supplementary Fig. 2).

Others have reported that type 1 diabetic subjects with autoimmune thyroid disease (15) or females (5,16) are more likely to be GADA-positive than those without thyroid disease and males,

respectively. Multivariate analysis, including onset and duration of disease, sex, and self-reported thyroid disease confirmed that GADA positivity is more likely in those of female sex (OR 1.41 [95% CI 1.25–1.59],  $P < 0.0001$ ) or with self-reported thyroid disease (1.41 [1.15–1.72],  $P = 0.001$ ). However, adjusting for sex and self-reported thyroid disease in the modified spline did not affect the significance of the interaction of onset and duration on GADA.

**CONCLUSIONS**—We explored the frequency of antibody positivity as a function of both age at diagnosis and duration of disease to understand whether these variables interacted; that is, whether the loss of antibody positivity over time is affected by the age at which the individual was diagnosed with type 1 diabetes. We found an interaction of onset and duration

Table 2—Multivariate logistic regression for GADA, IA-2A, and GADA- and/or IA-2A-positive subjects\*

Covariates	N	GADA			IA-2A			Aby <sup>+</sup>		
		% Pos	OR	95% CI	% Pos	OR	95% CI	% Pos	OR	95% CI
Onset age 2–7 years										
Duration (years)										
0–5	530	45.5	1	—	61.3	1	—	76.6	1	—
6–13	644	34.8	0.62†	0.49–0.80	44.6	0.46‡	0.36–0.59	62.3	0.47‡	0.36–0.61
≥ 14	565	27.6	0.44‡	0.34–0.58	24.2	0.16‡	0.12–0.21	42.7	0.20‡	0.15–0.26
Onset age 8–13 years										
Duration (years)										
0–5	745	58.8	1.73	1.37–2.19	65.1	1.17	0.91–1.50	82.1	1.39§	1.05–1.85
6–13	508	41.9	0.86	0.67–1.12	53.9	0.67§	0.51–0.87	71.9	0.74§	0.55–0.99
≥ 14	514	37.0	0.68§	0.52–0.88	35.0	0.30‡	0.23–0.39	56.2	0.36‡	0.27–0.48
Onset age ≥14 years										
Duration (years)										
0–5	567	70.5	2.99‡	2.30–3.89	53.4	0.73§	0.56–0.95	82.2	1.49§	1.09–2.03
6–13	389	65.3	2.25‡	1.69–3.00	42.7	0.42‡	0.31–0.56	73.8	0.82	0.60–1.13
≥ 14	558	42.5	0.90	0.70–1.15	26.2	0.20‡	0.15–0.26	53.4	0.34‡	0.26–0.45
HLA										
XX	374	36.6	1	—	30.2	1	—	50.0	1	—
DR3/X	627	49.6	1.94‡	1.42–2.65	26.0	0.85	0.62–1.16	57.3	1.50§	1.11–2.04
DR3/DR3	374	57.0	2.61‡	1.84–3.70	22.7	0.68§	0.47–0.99	65.2	2.11‡	1.49–2.99
DR3/DR4	1,847	47.9	2.03‡	1.54–2.69	49.1	2.75‡	2.10–3.61	70.2	3.14‡	2.39–4.13
DR4/X	1,278	41.8	1.40§	1.06–1.86	56.7	3.58‡	2.71–4.73	69.0	2.67‡	2.02–3.54
DR4/DR4	520	52.5	2.13‡	1.54–2.94	59.8	4.17‡	3.04–5.73	76.3	3.94‡	2.84–5.47

\*Shown are the results of three separate multivariate analyses with dependent variables GADA, IA-2A, or Aby<sup>+</sup>. Independent variables (covariate) are age of onset (split into tertiles), duration of disease (split into tertiles), and HLA type. Aby<sup>+</sup> indicates those who are GADA- and/or IA-2A-positive. % Pos, percentage of antibody-positive subjects; X ≠ DR3 or DR4. †P < 0.001; ‡P < 0.0001; §P < 0.05.

of diabetes on GADA positivity, but not on IA-2A positivity. Specifically, for individuals who were diagnosed with diabetes at age 14 or older, the percentage of individuals positive for GADA changed little during the first 5 years after diagnosis, in contrast to subjects diagnosed when they were younger than age 14, in which the percentage that remained GADA-positive fell rapidly during that time. The change in

antibody frequency was similar regardless of age of diagnosis during the next 8 years from onset of disease, only to decrease more rapidly in the older subjects long after diagnosis. Together with data indicating that GADA is the most frequent antibody reported in antibody-positive individuals with clinically apparent type 2 diabetes, our data suggest the hypothesis that there are differences in the biology

of GADA autoimmunity compared with other DAAs.

Because immune-mediated destruction of β-cells is suggested to occur more rapidly in younger than in older subjects (17), we postulated that there would be a more rapid decrease in the percentage of antibody-positive subjects over time from diagnosis among younger subjects. Yet, this was observed only

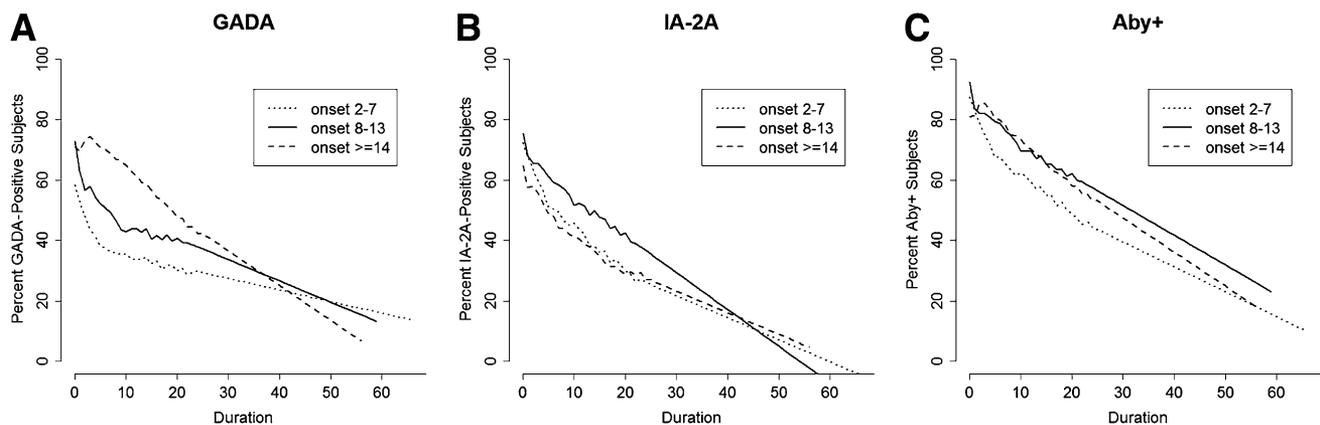
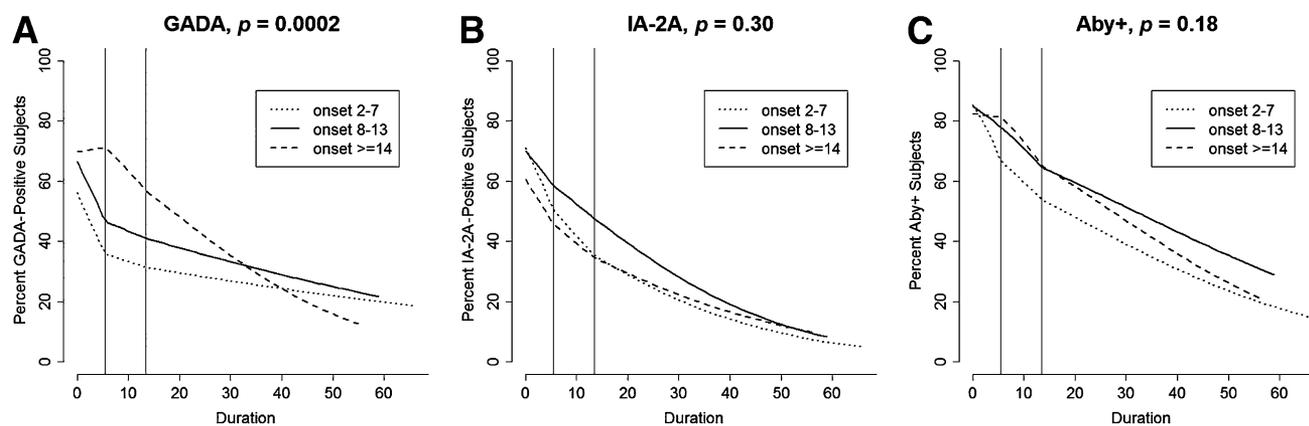


Figure 2—A smoothing function was used to plot the percentage of antibody-positive subjects within each tertile of onset, with duration as a continuous variable for GADA (A), IA-2A (B), and Aby<sup>+</sup> (C). Aby<sup>+</sup>, GADA- and/or IA-2A-positive.



**Figure 3**—Spline plots modeling onset and duration on the percentage of antibody-positive subjects. The interaction of onset and duration was significant for GADA (A) but not for IA-2A (B) or Aby<sup>+</sup> (C). P values for the interaction of onset and duration are shown at the top of each plot. The vertical lines at 5.5 years and 13.5 years indicate breakpoints in the spline, which is the division between the tertiles of duration. Aby<sup>+</sup>, GADA- and/or IA-2A-positive.

for GADA within 5 years of diagnosis, and not IA2.

Our data confirm the effect of age on the presence of autoantibodies, with younger subjects more likely to have IA-2A and older subjects more likely to have GADA. The notable exception to this statement is the high frequency of subjects with neither GADA nor IA-2A within the first 3 years of disease who were diagnosed when they were younger than age 2. Because samples were obtained after individuals had been treated with insulin, IAA were not measured. IAA are often found in younger subjects, so it is possible that some of these individuals do indeed have type 1a diabetes, which cannot be determined from this dataset. Alternatively, some of the antibody-negative subjects diagnosed at younger than age 2 may have monogenic diabetes. Recent reports indicate that about 1% of youth with diabetes have monogenic disease (18).

Several other findings extend previous reports. First, univariate analysis found that IA-2A were most common in subjects with onset at age 8 to 13, a finding similar to Graham et al. (5), who found that IA-2A was highest in individuals aged 7 to 13 years. However, multivariate analysis adjusting for duration failed to show a significant difference in the percent of IA-2A-positive subjects between those with onset at 2 to 7 years old and those with onset of 8 to 13 years old when duration was  $\leq 5$  years. Our findings of a positive association of IA-2A with DR4 and a negative association of IA-2A with DR3 are consistent with most previous reports. Despite this relationship, we found minimal to no impact

on the ORs for antibody status by age at diagnosis and duration of disease when HLA was included in the models.

Although cross-sectional and not longitudinal, with more than 5,000 individuals with type 1 diabetes, including a wide variation in their age of onset and the duration of disease at the time of blood sampling, this T1DGC dataset has considerable power to investigate the relationships described. Moreover, the data presented provide an important context for clinicians and investigators using autoantibody measurements to classify individuals with diabetes. With the caveat that this study measured only GADA and IA-2A, in clinical practice these are the most frequently ordered tests, either as a routine measure or to clarify diagnosis when the clinician is confronted with an “atypical” case, including an overweight child with diabetes or in cases of suspected monogenic diabetes.

Current guidelines recommend genetic screening for monogenic diabetes when various criteria are met, including the absence of DAAs (19). As can be seen from Table 1, within the first 5 years from diagnosis, about 20% of individuals, regardless of their age at diagnosis, had neither GADA nor IA-2A, and by 6 to 13 years after diagnosis, almost 40% were antibody-negative. These data serve as a reminder of the limitations of autoantibody screening for clinical classification in routine clinical care. Inappropriately interpreting a negative autoantibody test as ruling out type 1a diabetes may result in incorrect treatments, expensive genetic testing, and/or failure to recommend participation in autoantibody screening

studies to identify family members at risk for disease.

In summary, age at diagnosis and duration of disease both have an important impact on autoantibody status and should be considered when interpreting a result in individuals with diabetes. The impact of duration of disease on the frequency of IA-2A positivity was similar regardless of the age of diagnosis, whereas for GADA, individuals who were diagnosed at age 14 or older had a different pattern than subjects who were younger at the time of diagnosis. These data suggest that there may be differences in the underlying biology that results in measureable GADA versus IA-2A antibodies. Additional longitudinal studies would be required to confirm these findings.

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D.M.T. performed the analysis and wrote the manuscript. C.S. provided statistical consultation and code for various analyses, wrote the analysis section, and reviewed the manuscript. R.S.W. provided statistical consultation and code for various analyses and reviewed the manuscript. C.J.G. contributed to, reviewed, and edited the manuscript.

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