Autoantibodies to Truncated GAD(96-585) Antigen Stratify Risk of Early Insulin Requirement in Adult-Onset Diabetes

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We investigated whether characterization of full-length GAD (f-GADA) antibody (GADA) responses could identify early insulin requirement in adult-onset diabetes. In 179 f-GADA–positive participants diagnosed with type 2 diabetes, we assessed associations of truncated GADA (t-GADA) positivity, f-GADA IgG subclasses, and f-GADA affinity with early insulin requirement (<5 years), type 1 diabetes genetic risk score (T1D GRS), and C-peptide. t-GADA positivity was lower in f-GADA–positive without early insulin in comparison with f-GADA–positive type 2 diabetes requiring insulin within 5 years, and T1D (75% vs. 91% and 95% respectively, $P < 0.0001$). t-GADA positivity (in those f-GADA positive) identified a group with a higher T1D genetic susceptibility (mean T1D GRS 0.248 vs. 0.225, $P = 0.003$), lower C-peptide (1,156 pmol/L vs. 4,289 pmol/L, $P = 1 \times 10^{-7}$), and increased IA-2 antigen positivity (23% vs. 6%, $P = 0.03$). In survival analysis, t-GADA positivity was associated with early insulin requirement compared with those only positive for f-GADA, independently from age of diagnosis, f-GADA titer, and duration of diabetes (adjusted hazard ratio 5.7 [95% CI 1.4, 23.5], $P = 0.017$). The testing of t-GADA in f-GADA–positive individuals with type 2 diabetes identifies those who have genetic and clinical characteristics comparable to T1D and stratifies those at higher risk of early insulin requirement.

ARTICLE HIGHLIGHTS

- Progression to insulin therapy is highly variable in adult-onset GAD antibody (GADA)–positive diabetes.
- We further characterized GADA in adult-onset diabetes and assessed whether these are associated with early insulin requirement.
- Truncated GADA positivity was associated with a type 1 diabetes–like phenotype and stratified risk of early insulin requirement. Those GADA positive who were negative for truncated GADA had the characteristics and progression of classic type 2 diabetes. Assessing full-length GADA IgG subclass and affinity did not further stratify risk of progression.
- Truncated GADA assessment remains underused in clinical practice but could assist correct therapy allocation in adult-onset diabetes.

Autoantibodies to GAD (GADA) are common in adults initially diagnosed and treated as type 2 diabetes, with prevalence varying from 2 to >10%. depending on population and assay (1). This patient group, often described as having latent autoimmune diabetes (LADA), recently redefined by

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the World Health Organization (WHO) as slowly evolving immune-mediated diabetes (2,3), is highly heterogeneous, varying from those with very rapid progression to insulin therapy and a type 1 diabetes (T1D)–like phenotype, to those with the clinical course and characteristics of type 2 diabetes.

Whether this heterogeneity is best explained by an intermediate form of autoimmune diabetes or a mixture of autoimmune and nonautoimmune diabetes, due to the combination of imperfect islet autoantibody specificity and low prior likelihood of autoimmune diabetes (Bayes Theorem) in an adult population, or both, is a matter of debate (4–6). Approaches that improve specificity of GADA testing for identifying T1D would allow targeting of monitoring, advice, and early insulin initiation to those most likely to benefit.

Developments in assay technology allow measurement of additional characteristics beyond full-length GADA (f-GADA) titer, including epitope specificity, affinity, and IgG subclasses (7–9). The clinical utility of these GADA characteristics is unclear. Previous research in prediction has shown that GADA reactive to the N-terminally truncated GADA (GAD96-585; t-GADA) are more disease specific in first-degree relatives of patients with T1D, while maintaining sensitivity and specificity in newly diagnosed cases (8,10). Reactivity to t-GADA (11) and high f-GADA affinity (12) have been associated with risk of early insulin treatment in adult-onset diabetes, and increased IgG3 and IgG4 IgG subclasses have been associated with a slower rate of β-cell destruction in slow-evolving autoimmune diabetes (13).

We aimed to determine whether assessment of GADA truncated epitope specificity, affinity, and IgG subclasses in postdiagnosis f-GADA–positive type 2 diabetes, could improve the identification of patients with early insulin requirement.

RESEARCH DESIGN AND METHODS

Study Cohorts

Participants (N = 6,599) were included in this study if they had a clinical diagnosis of type 2 diabetes after age ≥18 and had no insulin requirement within 6 months of diagnosis. They were identified from six U.K. cohorts (recruited from primary and secondary care settings and had f-GADA assessed in the same laboratory) (14): the Genetics of Diabetes Audit and Research Tayside Study (GoDARTS) (15), Diabetes Alliance for Research in England (DARE) (16), Predicting Response to Incretin Based Agents in Type 2 Diabetes (PRIBA) (17), MRC MASTERMIND Progressors (18), and StartRight Studies (19,20).

In those found f-GADA positive (n = 179), we assessed f-GADA characteristics: t-GAD epitope specificity, f-GADA affinity, and f-GADA IgG subclass (Supplementary Table 1). We compared islet-autoantibody, genetic, and C-peptide characteristics with those of 6,420 participants with f-GADA–negative type 2 diabetes (clinical diagnosis and >6 months to insulin), and 141 participants with T1D (f-GADA positive, on insulin therapy from diagnosis, clinical diagnosis of T1D). Characteristics are summarized in Supplementary Tables 1–3.

Assessment of HbA_{1c} and Diabetes Progression (Time to Insulin)

Available HbA_{1c} at the latest follow-up (median diabetes duration 11 years [range 7–15]) was obtained from electronic health care records for the GoDARTS study (n = 3,893) or was measured on a research sample in recruitment centers' local laboratories (all are accredited National Health Service blood science laboratories) for the Exeter cohorts (PRIBA, MRC Progressors, StartRight, and DARE; n = 2,706).

For GoDARTS, time to insulin was defined from electronic prescription records. For Exeter cohorts (DARE, PRIBA, and MRC MASTERMIND Progressors), insulin treatment, date of commencing insulin, and date of diagnosis were self-reported at a single visit. For StartRight, insulin treatment, date of commencing insulin, and date of diagnosis were self-reported at three visits, within 12 months of diagnosis, and at ∼1 and 2 years later (21).

Laboratory Measurement of GADA to Full-Length GAD65(1-585)

Analysis of f-GADA was conducted at The Academic Department of Blood Sciences, Royal Devon and Exeter Hospital using the RSR Limited ELISA (Cardiff, U.K.) on the Dynex DS2 ELISA Robot (Dynex Technologics, Worthing, U.K.). The cutoff for positivity was ≥11 WHO units/mL, based on the 97.5th centile of 1,559 control participants without diabetes (22). In the 2020 International Islet Autoantibody Standardization Program (IASP2020), the assay specificity and adjusted sensitivity at 95% specificity (AS95) were 98.9% and 86%, respectively.

Assessment of GADA Characteristics

Of 6,599 participants, 179 (2.7%) were f-GADA positive with sera available for further characterization. These and 141 f-GADA–positive patients with T1D underwent further analysis to explore autoantibody characteristics: t-GADA epitope specificity, f-GADA affinity, and f-GADA IgG subclasses.

Measurement of GADA Epitope Specificity to Truncated GAD65(96-585)

t-GADA epitope specificity was determined by a luciferase immunoprecipitation system (LIPS) assay using nanoluciferase-tagged GAD65/67 kDa isoform of GAD antigen, with the N-terminal 95aa truncated (Nluc-GAD65[96-585]), as described previously, with the fluorimazine substrate diluted 1:3. Diabetic kidney (DK) units/mL were calculated using a logarithmic standard curve, and the threshold of positivity was ≥10.7 DK units/mL (based on the 97.5th centile of 221 healthy schoolchildren). In the IASP2020 workshop, the specificity and AS95 for this assay were 100% and 86%, respectively.

Measurement of GADA IgG Subclass Response to GAD65(1-585)

Determination of IgG subclasses to f-GADA was based on a previously published approach with modifications (9,23), described in detail in the Supplementary Methods.

Owing to serum availability, a subcohort of f-GADA–positive samples was selected for subclass analysis, ensuring an equal number of samples with f-GADA–positive type 2 diabetes, with and without progression to insulin within 5 years, and f-GADA–positive T1D. Samples from each cohort studied were run simultaneously in each assay, and where possible, were matched for f-GADA titer and affinity (closest available) across comparison groups.

Measurement of GADA Affinity to GAD65(1-585)

f-GADA affinity was measured by competitive binding experiments based on the approach developed by Mayr et al. (7), described in the Supplementary Methods.

The calculation of K_d values was limited to samples with IC_{50} values greater than the concentration of labeled GAD65 (1.88×10^{-10} mol/L). For samples with an IC_{50} of $<1.88 \times 10^{-10}$ mol/L, the f-GADA affinity of the sample was set at $K_d > 8 \times 10^{11}$ L/mol. A negative quality control sample (healthy adult) and a positive quality control sample (f-GADA–positive relative without diabetes [38% coefficient of variation (CV)]) was run alongside samples in each assay.

Assessment of IA-2 Antigen and Zinc Transporter 8 Autoantibodies Positivity

IA-2 antigen (IA-2A) and zinc transporter 8 autoantibodies (ZnT8A) assessment was undertaken in all samples from DARE, StartRight, MRC Progressors, PRIBA, and GoDARTS (ZnT8A only) and conducted on the same serum sample as the f-GADA assessment at The Academic Department of Blood Sciences, Royal Devon and Exeter Hospital using the RSR Limited ELISAs on the Dynex DS2 ELISA Robot. The cutoff for IA-2A positivity was ≥7.5 units/mL, based on the 97.5th centile of 1,559 control participants without diabetes (22). In the IASP2020 workshop, the specificity and AS95 was 98.9% and 72%, respectively. ZnT8A positivity was ≥65 WHO units/mL for those aged <30 years and ≥10 WHO units/mL for those aged ≥30 years, based on the 97.5th centile of 1,559 control participants without diabetes (24). In the IASP2020 workshop, the assay specificity and AS95 were 98.9% and 74%, respectively. Because IA-2A was not measured in GoDARTS, to ensure complete data for the f-GADA positives, IA-2A was remeasured on all 179 and the f-GADA positives with T1D, using a LIPS assay, as described above, but using a Nluc-tagged antigen specific to the intracytoplasmic (aa606-979) region of IA-2 (IA-2ic) provided by Vito Lampasona (Milan, Italy). The threshold of positivity was ≥0.3 DK units/mL (based on the 98th centile of 112 school children). In the IASP2020 workshop, the specificity and AS95 for this assay were 100% and 78%, respectively.

Additional Laboratory Analysis

Plasma C-peptide was measured, on a random nonfasting sample at a median of 12 years (range 4.6–40) after diagnosis, by electrochemiluminescence immunoassay (intra-assay CV, 3.3%; interassay CV, 4.5%) on a Roche Diagnostics (Mannheim, Germany) E170 analyzer by the Blood Sciences Department at the Royal Devon and Exeter NHS Foundation Trust (Exeter, U.K.).

We generated a weighted T1D genetic risk score (GRS) from 30 common T1D genetic variants (single nucleotide polymorphisms) for HLA and non-HLA loci as previously described (14,25).

Statistical Analysis

We compared proportions of the following GADA characteristics (t-GADA status [positive vs. negative], IgG subclass response [IgG1-restricted vs. IgG-unrestricted], and affinity [high vs. moderate/low affinity]) between f-GADA–positive clinically diagnosed type 2 diabetes, with and without early insulin treatment (<5 years), and f-GADA–positive T1D using the Pearson χ^2 test. We then assessed whether each characteristic was associated with clinical and biochemical participants' characteristics within all those with f-GADA–positive type 2 diabetes using Pearson χ^2 tests for proportions of categorical variables (IA-2A and ZnT8A positivity and early insulin requirement [<5 years]) and *t* tests for continuous variables (C-peptide, T1D GRS, f-GADA titer, and age at diagnosis).

We assessed the relationship between GADA characteristics and progression to insulin (censored at 5 years) using Cox proportional hazard models (after confirming model assumptions) in univariable and multivariable models, with adjustment for f-GADA titer, duration of diabetes at f-GADA test, and age at diagnosis. Sex was not adjusted for or considered a factor in the statistical analysis. For f-GADA affinity, we also assessed whether there was an association between higher affinity and progression to insulin therapy independent of t-GADA specificity in addition to the above covariates. All statistical analysis was done using Stata/SE 16.0 (StataCorp, College Station, TX), unless otherwise stated, and graphed using GraphPad Prism3.

Data and Resource Availability

The StartRight data set generated during and/or analyzed in the current study is available from the corresponding author upon reasonable request. Data pertaining to the other Exeter studies (DARE, PRIBA and MRX Progressors) can be accessed via application to the Peninsula Research Bank, and for GoDARTS via application to the GoDARTS study committee. No applicable resources were generated or analyzed during the current study.

RESULTS

In those with f-GADA–positive type 2 diabetes (*n* = 179), median follow-up was 12 years, with f-GADA assessment

at a median of 4.9 years' diabetes duration; 35% (n = 63) of participants had progressed to insulin ≤5 years. In the comparison cohorts, those with f-GADA–negative type 2 diabetes (n = 6,420) and f-GADA–positive T1D (n = 141) had a median follow-up 11 and 15 years, and f-GADA assessment was at a median 5.6 and 16 years' diabetes duration, respectively.

Participants With Positive GADA for a Truncated Epitope Have Enrichment for Genetic and Clinical Characteristics Associated With T1D

Positivity for t-GADA was similar between individuals with f-GADA–positive T1D and those with f-GADA–positive type 2 diabetes requiring early insulin (≤5 years) 95% (95% CI 90, 98) vs. 97% (95% CI 89, 100) respectively (P = 0.57). In contrast, the proportion of t-GADA positivity in those without early insulin requirement was significantly lower (72% [95% CI 63, 80]) than individuals with early insulin requirement (97%, $P = 7 \times 10^{-5}$) and the T1D cohort (95%, $P = 4 \times 10^{-7}$) (Fig. 1*A*). t-GADA positivity identified a group diagnosed younger (mean 55 years [95% CI 52, 57] vs. 62 years [95% CI 58, 66], P = 0.002), with a higher T1D GRS (mean 0.248 [95% CI 0.241, 0.254] vs. 0.225 [95% CI 0.213, 0.237], P = 0.003), lower C-peptide levels (mean 1,155 pmol/L [95% CI 918, 1,393] vs. 4,289 pmol/L [95% CI 845, 7,732], $P = 1 \times 10^{-7}$) at a median duration of 12 years at C-peptide testing (Supplementary Fig. 1*A–D*) and increased positivity for IA-2A (23% [95% CI 17, 31] vs. 6% [95% CI 0.7, 19.7], P = 0.022) and ZnT8A (21% [95% CI 14, 28] vs. 0% [95% CI 0, 10], P = 0.004) (Table 1).

t-GADA Epitope Positivity Is Independently Associated With Increased Risk of Early Insulin Therapy

In survival analysis t-GADA positivity (in those f-GADA positive) identified participants at higher risk of early insulin requirement compared with those f-GADA positive and t-GADA negative (hazard ratio [HR] 8.4 [95% CI 2.1, 34.4], P = 0.003) (Table 2 and Fig. 2*A*). The association between t-GADA positivity (in those f-GADA positive) and early insulin requirement persisted after adjustment for age at diagnosis, f-GADA titer, and duration at GADA testing (adjusted HR 5.7 [95% CI 1.4, 23.5], P = 0.017) compared with those f-GADA positive and t-GADA negative (Table 2). Findings were also similar with additional adjustment for presence of IA-2 and/or ZnT8 autoantibodies (HR 6.1 [95% CI 3.9, 9.5], $P < 0.001$) (Supplementary Table 4). Those positive for f-GADA but negative for t-GADA had similar risk of progression to early insulin requirement compared with those with f-GADA–negative type 2 diabetes (HR 0.93 [95% CI 0.23, 3.72], P = 0.9). This was similar after adjustment for age at diagnosis, f-GADA titer, and diabetes duration at GADA testing (adjusted HR 0.98 [95% CI 0.24, 3.95], P = 0.98) (Supplementary Table 4).

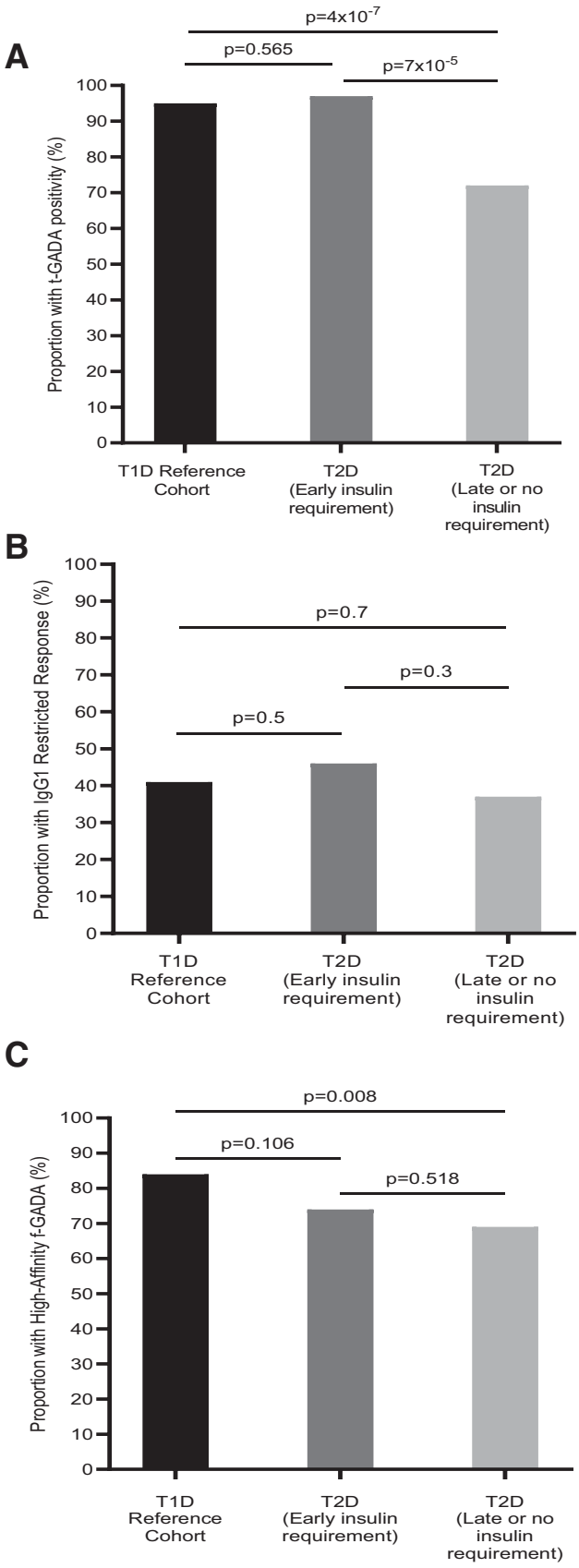

Figure 1—Proportions of individuals with t-GADA (*A*), IgG1-restricted f-GADA response (*B*), and high-affinity f-GADA response (*C*). T2D, type 2 diabetes.

Table 1—Diabetes characteristics comparison between those positive and negative for t-GADA in those f-GADA positive

	f-GADA and t-GADA positive (n = 145)	f-GADA positive and t-GADA negative (n = 34)	t-GADA positive vs. t-GADA negative P value	f-GADA positive T1D (n = 141)	f-GADA negative (n = 6,420)
Age at diagnosis (years)	54.6 (52.4, 56.8)	62.3 (58.5, 66.1)	0.002	27.1 (24.4, 29.7)	60.4 (60.1, 60.6)
f-GADA titer (WHO units/mL)	771 (631, 910)	227 (65.3, 388)	0.0004	622 (488, 755)	NA
T1D GRS	0.248 (0.241, 0.254)	0.225 (0.213, 0.237)	0.003	0.274 (0.269, 0.279)	0.228 (0.227, 0.229)
C-peptide (pmol/L)	1,156 (918, 1393)	4,289 (845, 7,732)	<0.0001	54.2 (29.4, 78.9)	2,369 (2,283, 2,454)
IA-2A positive	33 (23; 16, 30)	3 (5.9; 0.72, 19.7)	0.026	70 (50; 41, 58)	15 (0.6; 0.3, 0.9)*
ZnT8A positive	30 (21; 14, 28)	0 (0; 0, 10.3)	0.004	49 (39; 30, 48)	28 (1.7; 1.2, 2.5)†
Insulin treated within 5 years	61 (42; 34, 51)	2 (5.9; 0.72, 19.7)	<0.0001	141 (100; 100, 100)	429 (6.7; 6.1, 7.3)

Data displayed as n (%; 95% CI) or mean (95% CI). *Of 2,607 tested. †Of 1,615 tested.

f-GADA IgG Subclasses Do Not Identify Those at Risk of Early Insulin Therapy

The prevalence of each f-GADA IgG subclass was similar between f-GADA–positive type 2 diabetes participants with and without early insulin requirement and those with f-GADA–positive T1D ($P > 0.07$ for all comparisons) (Supplementary Table 5). The rank order of frequencies of IgG subclasses was the same between those with type 2 diabetes and early insulin requirement and those without (IgG1 > IgG3 > IgG2 > IgG4). In the f-GADA–positive T1D reference group, the rank order of frequencies of IgG subclasses was IgG1 > IgG3 > IgG4 > IgG2. f-GADA IgG subclasses were unable to be detected in 13 (6%) of the subset tested. Because IgG1 was the most common IgG subclass in all three cohorts, we split the cohort into two response categories for further analysis: IgG1 only (IgG1-restricted) versus IgG1 plus other IgG subclasses (IgG-unrestricted). The proportion of those with an IgG1-restricted response was similar between those with type 2 diabetes and early insulin requirement versus those without (42% [95% CI 29, 57] vs. 39% [95% CI 28, 52], $P = 0.7$). The proportion of those with an IgG1-restricted response in the f-GADA–positive T1D group was similar (40% [95% CI 29, 53], P vs. other subgroups >0.8) (Fig. 1*B*). IgG subclass response was not associated with clinical characteristics (age at diagnosis, T1D GRS, C-peptide levels, and IA-2A and ZnT8A positivity), but those with an IgG1-restricted response had lower levels of f-GADA than those with an IgG-unrestricted response (mean 468 WHO units/mL [95% CI 283, 652] vs. 1,130 WHO units/mL [95% CI 918, 1342], $P < 0.0001$) (Supplementary Table 6).

In survival analysis, an IgG1-restricted response did not identify those at risk for early insulin requirement in those that were f-GADA positive (HR 1.07 [95% CI 0.62, 1.9], $P = 0.8$) (Fig. 2*B*). This was still the case when the model was adjusted for age at diagnosis and duration of diabetes (HR 1.02 [95% CI 0.58, 1.8], $P = 0.9$) (Supplementary Table 7). The presence of each individual IgG subclass was not associated with progression to insulin in survival analysis (Supplementary Table 8).

Proportion of High-Affinity f-GADA Was Lower in Those With Type 2 Diabetes

The affinities of f-GADA detected ranged from 7.57×10^6 to $>8 \times 10^{11}$ L/mol across all groups (type 2 diabetes with early insulin requirement: 3.94×10^7 to $>8 \times 10^{11}$ L/mol; type 2 diabetes with no/later insulin requirement: 7.57×10^6 to $>8 \times 10^{11}$ L/mol; and f-GADA–positive T1D: 3.76×10^7 to $>8 \times 10^{11}$ L/mol). For categorical analysis, affinities were split into high-affinity ($\geq 1 \times 10^9$ L/mol) and moderate-/low-affinity ($<1 \times 10^9$ L/mol) groups, in line with previous publications (7,12,26). The proportion of

Table 2—HRs from Cox proportional regression models for time to insulin censored at 5 years

Survival analysis t-GADA positive vs. t-GADA negative (in those f-GADA positive)	Unadjusted model		Adjusted model	
	HR (95% CI)	P value	HR (95% CI)	P value
t-GADA negative (reference)	1		1	
t-GADA positive (vs. reference)	8.4 (2.05, 34.4)	0.003	5.7 (1.4, 23.5)	0.017
Age of diagnosis (per 10-year increase)			0.94 (0.92, 0.96)	<0.001
f-GADA titer (per 100-unit increase)			1.01 (0.98, 1.04)	0.536
Diabetes duration at f-GADA testing (per 1-year increase)			0.88 (0.83, 0.94)	<0.001

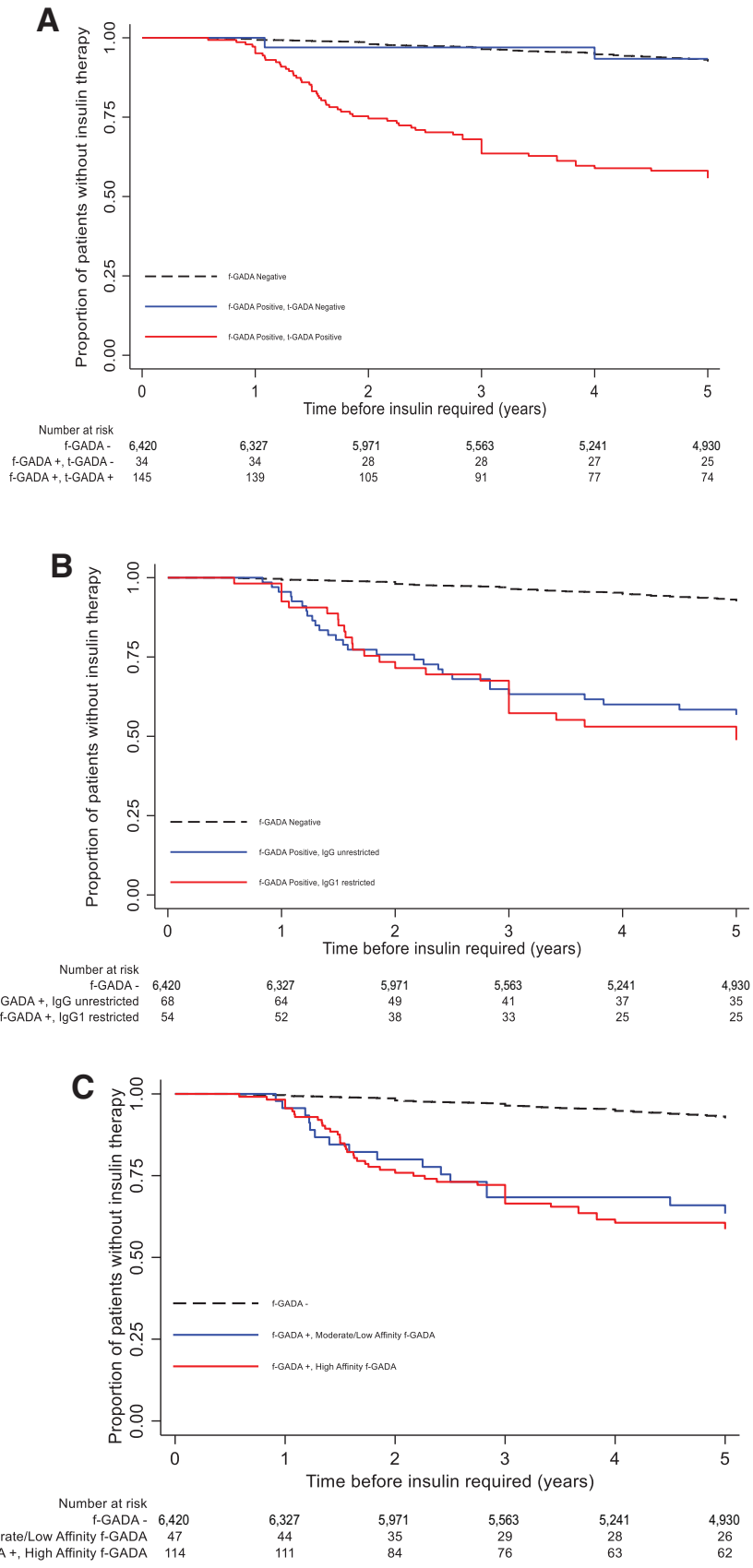

Figure 2—Kaplan-Meier plots of probability of requiring insulin therapy during 5-year follow-up in those clinically diagnosed with type 2 diabetes, stratified by risk group of f-GADA and t-GADA positivity (*A*), by risk group of f-GADA positivity and subclass (*B*), and by risk group of f-GADA positivity and affinity (*C*).

those with high-affinity f-GADA was similar between those with type 2 diabetes with and without early insulin requirement (74% [95% CI 61, 84) vs. 69% [95% CI 59, 78], P = 0.5). Those with f-GADA–positive T1D had a higher proportion of those with high-affinity f-GADA (84% [95% CI 76, 89]) compared with those with (P = 0.1) and without (P = 0.008) early insulin requirement (Fig. 1*C*). There were no differences in age at diagnosis, C-peptide levels, and IA-2A and ZnT8A positivity between those with high and moderate/low affinity (Supplementary Table 9). However, those with high-affinity f-GADA had lower f-GADA titers (mean 546 WHO units/mL [95% CI 409, 683] vs. mean 1,167 WHO units/mL [95% CI 902, 1432], $P = 1 \times 10^{-5}$) and higher T1D GRS (mean 0.249 [95% CI 0.242, 0.256] vs. mean 0.232 [96% CI 0.219, 0.244], P = 0.01) than those with moderate-/low-affinity f-GADA.

Stratification by f-GADA affinity category in those f-GADA positive did not stratify risk of progression to insulin therapy (HR 1.13 [95% CI 0.64, 2.01], P = 0.66) (Fig. 2*C*). Again, this was still the case when the model was adjusted for age at diagnosis, f-GADA titer, and duration of diabetes at f-GADA testing (HR 1.17 [95% CI 0.63, 2.17], P = 0.62) (Supplementary Table 10). f-GADA affinity did not further stratify early insulin requirement in those found to be t-GADA positive (Supplementary Fig. 2).

DISCUSSION

Our study shows that in individuals with f-GADA–positive type 2 diabetes, testing for t-GADA identified those with a more T1D-like phenotype (diagnosed younger, increased proportion positive for multiple islet autoantibodies, increased T1D GRS, and lower C-peptide levels) and stratifies risk of early insulin requirement. Although t-GADA positivity is strongly associated with early insulin requirement, participants positive for f-GADA but negative for t-GADA had similar risk of early insulin requirement to f-GADA–negative type 2 diabetes. In contrast, assessment of f-GADA affinity and IgG subclass response did not further stratify risk of early insulin requirement over and above f-GADA testing and (with the exception of affinity and T1D GRS) were not associated with other characteristics of T1D.

We have shown for the first time that t-GADA identified those at risk for early insulin requirement independently of f-GADA titer, duration of diabetes at GADA assessment, and age at diagnosis. This is the first study to assess relationships between t-GADA epitope, f-GADA affinity, and IgG subclass response (in the same cohort) with early insulin treatment using survival analysis, and the first to compare these characteristics in a large cohort with f-GADA positivity assessed using a highly specific, clinically available assay. This is a highly unique cohort because we had follow-up C-peptide data from diagnosis as well as T1D GRS.

Our finding of positivity for t-GADA, identifying those at higher risk of early insulin requirement, with a more T1D-like phenotype (younger at diagnosis, leaner, and with more diabetes-associated autoantibodies) (Table 1 and Supplementary Fig. 1) is consistent with Achenbach et al. (11); however, the utility of t-GADA in predicting early insulin using survival analysis (in a cohort with longer follow-up) and the association with higher T1D GRS and lower C-peptide were not previously described. Overall, the proportion of those progressing to insulin therapy in f-GADA–positive but t-GADA–negative cases (5.9%) was similar to f-GADA–negative cases (6.7%). In the study by Achenbach et al. (11), there was similarly no evidence of excess insulin therapy in f-GADA–positive t-GADA–negative participants compared with f-GADA–negative participants; indeed, association with insulin therapy was numerically but not statistically lower in the restricted full-length positive group: 13.7% (f-GADA positive, t-GADA negative) vs. 21.5% (f-GADA negative, t-GADA negative).

In line with previous studies, we found that IgG1 was the most dominant IgG subclass present in those with diabetes, regardless of diabetes classification, and that the presence of other IgG subclasses increased with increasing titer of f-GADA autoantibodies (9,23,27,28). f-GADA IgG subclass did not predict early insulin requirement in our cohort, similar to the lack of association between risk of T1D and f-GADA IgG subclasses in first-degree relatives observed by Achenbach et al. (23). Like Hillman et al. (13), all IgG subclasses of f-GADA were present in our adult-onset cohort, initially diagnosed with type 2 diabetes, with similar proportions observed for IgG1, IgG2, and IgG4. We observed some minor differences of higher IgG3 in type 2 diabetes and IgG4 in T1D. This may be due to the IgG3 clone used in our study and the longer duration of diabetes at sampling, respectively (29).

We have shown that higher affinity f-GADA does not identify higher risk of early insulin requirement or a more T1D-like phenotype, in contrast to previous research (12,30). This may be due to the wide variation in affinities found in the studies, differences in f-GADA screening and affinity assay format, and differences in duration of diabetes at testing or to differences in what is described as higher or lower affinity.

A strength of our study is the size and detailed follow-up of the initial adult-onset cohort with type 2 diabetes (>6,000) screened for f-GADA in one laboratory, using a highly robust and specific bridge ELISA assay and a positivity threshold based on a large control population. We were also able to apply a series of well-developed strategies and high-quality tests to examine in detail the characteristics of GADA in this well-defined cohort and compare a cohort with f-GADA–positive T1D. To improve upon the clinical ELISA assay (used as the f-GADA screen in this study), future work could try to incorporate the N-terminally truncated assay into the plate format, because although the t-GADA LIPS assay can be used for screening in a research setting, it is not set up on an automated platform.

A caveat of our research is that t-GADA testing was applied only to participants positive for a f-GADA assay due to time, sample availability, and cost constraints. Because

we have not tested those negative for the full-length assay, our results can only currently be applied to those who have previously tested f-GADA positive, and findings for the whole cohort (including f-GADA–negative participants) should be treated with caution. It is possible that false positive results for t-GADA could occur in the >6,000 of our cohort not tested for t-GADA, which could blunt the diagnostic accuracy and HRs of t-GADA testing reported for the whole cohort in this study. Previously, Williams et al. (8) identified 1% of those who previously tested f-GADA negative to be t-GADA positive. Such low rates of t-GADA positivity in those who are f-GADA negative could lend support to the assumption that t-GADA is likely to have high specificity when applied to a whole population.

The f-GADA characterization assays described here were conducted in different assay formats to the initial screening assay. The original f-GADA screen used the RSR ELISA assay, whereas the f-GADA characteristics were conducted using liquid-phase radiobinding and LIPS assays. Differences between ELISA and liquid-phase assays have been reported to impact specificity and sensitivity (31,32). IgG subclasses, epitope, and affinity characteristics cannot be assessed via RSR bridging ELISA assays. Because the RSR bridging ELISA is the most commercially and clinically used f-GADA assay, with the highest overall performance in IASP (33), this allows us to compare the t-GADA LIPS assay with a highly specific and currently used assay in the clinical setting. A further limitation is that f-GADA positivity was assessed in all patients with type 2 diabetes at a median 5.6 years after diabetes diagnosis; thus, f-GADA prevalence is likely to be lower than at diagnosis, although in adult-onset diabetes differences at this duration are modest (14,34).

Diagnosing autoimmune diabetes in later life is an important and challenging clinical problem, and f-GADA assays are unlikely to be sufficiently specific to confirm autoimmune diabetes in the setting of those diagnosed initially with type 2 diabetes (4,6,35). Therefore, approaches that improve islet autoantibody test specificity are needed to improve identification of autoimmune diabetes in adults (4). Our findings suggest that t-GADA assays may improve identification of patients with early progression and a T1D phenotype, potentially improving clinical outcomes and providing support for t-GADA testing replacing or adding to f-GADA testing in a clinical setting. Assays for f-GADA affinity or IgG subclass are more expensive, require specialist reagents and techniques, and do not lend themselves readily to testing in a clinical setting. Our findings that neither affinity nor IgG subclass differed between those with early and slow/no insulin progression and did not stratify risk of early insulin requirement in survival analysis suggests that they are unlikely to improve prediction of risk of early insulin requirement, compared with testing for t-GADA and suggests they are unlikely to have clinical utility for this purpose.

In conclusion, the testing of t-GADA in f-GADA–positive individuals with type 2 diabetes identifies those who have genetic and clinical characteristics comparable to T1D and stratifies those at higher risk of early insulin requirement.

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