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Title: Autoantibodies to truncated GAD(96-585) antigen stratify risk of early insulin requirement in adult-onset diabetes

Short Running Title: Utility of GADA characterisation in diabetes

Authors: Sian L. Grace[1,2] (sian.grace@bristol.ac.uk), Kathleen M. Gillespie[2] (k.m.gillespie@bristol.ac.uk), Claire L. Williams[2] (claire.l.williams@bristol.ac.uk), Vito Lampasona[3] (lampasona.vito@hsr.it), Peter Achenbach[4,5] (peter.achenbach@helmholtz.munich.de), Ewan R. Pearson[6] (E.Z.Pearson@dundee.ac.uk), Alistair J.K. Williams[2†], Anna E. Long[2*] (anna.long@bristol.ac.uk), Timothy J. McDonald[1,7*] (timothy.mcdonald@nhs.net), Angus G. Jones[1,8*] (Angus.Jones@exeter.ac.uk)

Author Affiliations:

[1]The Department of Biological and Clinical Science, University of Exeter Medical School, Exeter, U.K. [2]School of Translational Sciences, Bristol Medical School, University of Bristol, Bristol, U.K. [3]San Raffaele Diabetes Research Institute, IRCCS San Raffaele Scientific Institute, Milan, Italy. [4]Institute of Diabetes Research, Helmholtz Munich, German Center for Environmental Health, Munich, Germany. [5]Technical University of Munich, School of Medicine, Klinikum rechts der Isar, Forschergruppe Diabetes, Munich, Germany. [6]Biomedical Research Institute, University of Dundee, Dundee, U.K. [7]Academic Department of Clinical Biochemistry, Royal Devon and Exeter NHS Foundation Trust, Exeter, U.K. [8]Macleod Diabetes and Endocrine Centre, Royal Devon and Exeter NHS Foundation Trust, Exeter, U.K.

[*]AEL, TJM and AGJ are joint senior authors

Corresponding author: Professor Angus G. Jones. Email: angus.jones@exeter.ac.uk Telephone: +4401392 408538 Address: University of Exeter Medical School, RILD Building, RD&E Hospital, Wonford, Barrack Road, Exeter, EX2 5DW.

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Abstract

We investigated whether characterisation of full-length (f-)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 (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 to f-GADA positive type 2 diabetes requiring insulin within 5 years, and type 1 diabetes (75% vs. 91% and 95% respectively, p<0.0001). t-GADA positivity (in those f-GADA positive) identified a group with a higher type 1 diabetes genetic susceptibility (mean T1D GRS 0.248 vs. 0.225, p=0.003), lower C-peptide (1156 pmol/L vs. 4289 pmol/L, $p=1x10^{-7}$), and increased IA-2A 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 titre and duration of diabetes [adjusted HR 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 type 1 diabetes and stratifies those at higher risk of early insulin requirement.

Article Highlights

- Progression to insulin therapy is highly variable in adult-onset GADA positive diabetes.
- We further characterised 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.

- t-GADA assessment remains underutilised in clinical practice, but could assist correct therapy allocation in adult-onset diabetes.

Autoantibodies to GAD 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 the 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 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 non-autoimmune 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 type 1 diabetes 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 (f-)GADA titre, 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 GAD antigen (GAD96-585; t-GADA) are more disease-specific in first-degree relatives

of patients with type 1 diabetes, whilst 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 beta cell destruction in slow-evolving autoimmune diabetes (13).

We aimed to determine whether assessment of GADA truncated epitope specificity, affinity, and IgG subclasses in post diagnosis 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 6 UK 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 (Supplemental Table 1). We compared islet-autoantibody, genetic and C-peptide characteristics with those 6,420 participants with f-GADA negative type 2 diabetes (clinical diagnosis and >6 months to insulin), and 141 participants with type 1 diabetes (f-GADA positive, on

insulin therapy from diagnosis, clinical diagnosis of type 1 diabetes). Characteristics shown in Supplemental Tables 1-3.

Assessment of HbA1c and Diabetes Progression (Time to Insulin)

Available HbA_{1c} at 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 NHS blood science laboratories) for the Exeter cohorts (PRIBA, MRC Progressors, StartRight, DARE; n=2,706).

For GoDARTS, time to insulin was defined from electronic prescription records. For Exeter cohorts (DARE, PRIBA, 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 approximately 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 cut-off for positivity was ≥11 World Health Organisation (WHO) units/mL, based on the 97.5^{th} centile of 1,559 control participants without diabetes (22). In the 2020 International Islet Autoantibody Standardization Program (IASP) 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 characterisation. These, and 141 f-GADA positive patients with type 1 diabetes, 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 units/ml (DK U/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 ESM Supplemental Methods.

Due to serum availability, a sub-cohort of f-GADA positive samples were 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 type 1 diabetes. Samples from each cohort studied were run simultaneously in each assay, and where possible, were matched for f-GADA titre 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 ESM Supplemental Methods.

The calculation of K_d values was limited to samples with IC_{50} values greater than the concentration of labelled GAD65 ($1.88x10^{-10}$ mol/l). For samples with an IC_{50} <$1.88x10^{-10}$ mol/L, the f-GADA affinity of the sample was set at K_d >$8x10^{11}$ l/mol. A negative QC sample (healthy adult) and a positive QC sample [f-GADA positive relative without diabetes, (38% CV)] was run alongside samples in each assay.

Assessment of IA-2A & ZnT8A Positivity

IA-2A & 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 cut-off for IA-2A positivity was ≥7.5 units/mL, based on the 97.5^{th} 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 97.5^{th} centile of 1,559 control participants without diabetes (24). In the IASP2020 workshop, the assay specificity and AS95 were 98.9% and 74%, respectively. As 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 type 1 diabetes, using a LIPS assay, as described above, but using a Nluc-tagged antigen specific to the intracytoplasmic (aa606-979) region of islet antigen-2 (IA-2ic) kindly provided by Vito Lampasona (Milan, Italy). The threshold of positivity was ≥0.3 DK U/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 non-fasting sample at median 12 years (range 4.6-40 years) post diagnosis, by electrochemiluminescence immunoassay (intra-assay CV, 3.3%; inter-assay CV, 4.5%) on a Roche Diagnostics (Mannheim, Germany) E170 analyser by the Blood Sciences Department at the Royal Devon and Exeter NHS Foundation Trust (Exeter, U.K.)

We generated weighted T1D-GRS from 30 common type 1 diabetes genetic variants [single nucleotide polymorphisms (SNPs)] 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 type 1 diabetes using the Pearson chi-squared 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 chi-squared tests for proportions of categorical variables [IA-2A & ZnT8A positivity and early insulin requirement (<5 years)] and t-tests for continuous variables (C-peptide, T1D-GRS, f-GADA titre 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 titre, 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 co-variates. All statistical analysis was carried out using Stata/SE 16.0 (StataCorp, College Station, TX) unless otherwise stated and graphed using GraphPad Prism3.

Data and Resource Availability

The StartRight dataset generated during and/or analysed 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 Penninsula 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 type 1 diabetes (n=141); median follow-up was 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 type 1 diabetes

Positivity for t-GADA was similar between individuals with f-GADA positive type 1 diabetes 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%, $p=7x10^{-5}$) and the type 1 diabetes cohort (95%, $p=4x10^{-7}$)) (Figure 1A). 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 1155 pmol/L (95% CI 918, 1393) vs. 4289 pmol/L (95% CI 845, 7732), $p=1x10^{-7}$ at a median duration of 12 years at C-peptide testing] (Supplemental Figure 1A-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).

Truncated 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 to those f-GADA positive & t-GADA negative [HR 8.4 (95% CI 2.1, 34.4), p=0.003] (Table 2; Figure 2A). The association between t-GADA positivity (in those f-GADA positive) and early insulin requirement persisted after adjustment for age-at- diagnosis, f-GADA titre and duration at GADA testing [adjusted HR 5.7 (95% CI 1.4, 23.5), p=0.017] compared to 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] (Supplemental Table 4). Those positive for f-GADA but negative for t-GADA had similar risk of progression to early insulin requirement compared to

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 titre and diabetes duration at GADA testing [adjusted HR 0.98 (95% CI 0.24, 3.95), p=0.98] (Supplemental Table 4).

Full-length 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 type 1 diabetes (p>0.07 for all comparisons, Supplemental 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 type 1 diabetes 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. As 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) vs. IgG1 + 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 vs. 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 type 1 diabetes group was similar [40% (95% CI 29, 53), p vs. other subgroups >0.8] (Figure 1B). IgG subclass response was not associated with clinical characteristics (age-at-diagnosis, T1D-GRS, c-peptide levels, and IA-2A & 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 U/ml (95% CI 283, 652) vs. 1130 WHO U/ml (95% CI 918, 1342), p<0.0001 (Supplemental Table 6).

In survival analysis, an IgG1-restricted response did not identify those at risk of early insulin requirement in those that were f-GADA positive [HR 1.07 (95% CI 0.62, 1.9), p=0.8] (Figure 2B). 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] (Supplemental Table 7). The presence of each individual IgG subclass was not associated with progression to insulin in survival analysis (Supplemental Table 8).

The proportion of high affinity full-length GADA was lower in those with type 2 diabetes

The affinities of f-GADA detected ranged from $7.57x10^6$ to >8 $x10^{11}$ l/mol across all groups (type 2 diabetes with early insulin requirement $3.94\ x10^7$ to >8 $x10^{11}$ l/mol, type 2 diabetes with no/later insulin requirement $7.57\ x10^6$ to >8 $x10^{11}$ l/mol, f-GADA positive type 1 diabetes $3.76x10^7$ to >8 $x10^{11}$ l/mol). For categorial analysis, affinities were split into high (≥$1x10^9$ l/mol) and moderate/low affinity groups (<$1x10^9$ l/mol) in line with previous publications (7; 12; 26). The proportion of 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 type 1 diabetes had a higher proportion of those with high affinity f-GADA [84% (95% CI 76, 89)] compared to those with early insulin requirement (p=0.1) and without (p=0.008) (Figure 1C). There were no differences in age-at-diagnosis, c-peptide levels, and IA-2A & ZnT8A positivity between those with high and moderate/low affinity (Supplemental Table 9). However, those with high affinity f-GADA had lower f-GADA titres [mean 546 WHO U/ml (95% CI 409, 683) vs. mean

1167 WHO U/ml (95% CI 902, 1432), $p=1x10^{-5}$] 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] (Figure 2C). Again, this was still the case when the model was adjusted for age-at-diagnosis, f-GADA titre, and duration of diabetes at f-GADA testing [HR 1.17 (95% CI 0.63, 2.17), p=0.62] (Supplemental table 10). f-GADA affinity did not further stratify early insulin requirement in those found to be t-GADA positive (Supplemental Figure 2).

Conclusions

Our study shows that in individuals with f-GADA positive type 2 diabetes, testing for t-GADA identified those with a type 1 diabetes 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. Whilst 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 type 1 diabetes.

We have shown for the first time that t-GADA identified those at risk of early insulin requirement independently of f-GADA titre, 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 as 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 type 1 diabetes-like phenotype (younger at diagnosis, leaner and with more diabetes-associated autoantibodies [Table 1 & Supplemental Figure 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), similarly there was no evidence of excess insulin therapy in f-GADA positive t-GADA negative participants in comparison to f-GADA negative; 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 titre 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 type 1 diabetes 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, higher IgG3 in type 2 diabetes and IgG4 in type 1 diabetes. This may be due to the IgG3 clone utilized in our study and the longer duration of diabetes at sampling, respectively (29).

We have shown that higher affinity f-GADA do not identify higher risk of early insulin requirement or a more type 1 diabetes-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, 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 type 1 diabetes. 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, as whilst 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. As we have not tested those negative for the full-length assay, our results can only currently be applied to those that 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 >6000 of

our cohort not tested for t-GADA which could blunt the diagnostic accuracy and hazard ratios of t-GADA testing reported for the whole cohort in this study. Previously, Williams *et al*, identified 1% of those that previously tested f-GADA negative to be t-GADA positive (8). 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 characterisation assays described here were conducted in different assay formats to the initial screening assay. The original f-GADA screen used the RSR ELISA assay, whilst 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. As the RSR bridging ELISA is the most commercially and clinically used f-GADA assay (with 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, 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 as 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 type 1 diabetes 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, requiring specialist reagents and techniques and do not lend themselves readily to testing in a clinical setting. Our findings that neither affinity or 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 to testing for t-GADA, 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 type 1 diabetes and stratifies those at higher risk of early insulin requirement.

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Conflict of Interest

There are no conflicts of interest to report from the authors.

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KMG, AJKW (deceased), TJM and AGJ initially designed the study. ERP, TJM and AGJ created and managed the research studies. SLG, AEL and CLW researched the GADA characteristic data using specialist assays created, developed and optimised by CLW, AJKW, VL and PA. SLG, AEL, KMG, TJM and AGJ analysed the data with

advice from CLW and PA. SLG wrote the first draft of the manuscript with AEL, KMG, TJM and AGJ. All authors provided helpful discussion and reviewed and edited the manuscript. AGJ is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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This data has been presented in part at the following conferences:

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	f-GADA & t-GADA positive	f-GADA positive & t-GADA negative	t-GADA positive vs. t-GADA negative *p* value	f-GADA positive Type 1 Diabetes	f-GADA negative
n	145	34		141	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 Titre (WHO U/ml)	771 (631, 910)	227 (65.3, 388)	0.0004	622 (488, 755)	5.5 (5.4, 5.5)
T1D Genetic Risk Score	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)	1156 (918, 1393)	4289 (845, 7732)	<0.0001	54.2 (29.4, 78.9)	2369 (2283, 2454)
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)

Table 1: Diabetes characteristics comparison between those positive and negative for t-GADA in those f-GADA positive. Data displayed as n(%; 95% CI) or mean (95% CI). *Out of 2,607 tested. †Out of 1,615 tested. t-GADA; truncated GAD(96-585) autoantibody. f-GADA; full length GAD(1-585) autoantibody. IA-2A; islet antigen-2 autoantibody. ZnT8A; zinc transporter 8 autoantibody.

Survival analysis t-GADA pos vs t-GADA neg (In those f-GADA positive)	**Unadjusted Model**		**Adjusted Model**	
	Hazard ratio (95% CI)	***p value***	***Hazard ratio (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 1 year increase)			0.94 (0.92, 0.96)	<0.001
f-GADA Titre (per 100 unit increase)			1.01 (0.98, 1.04)	0.536
Duration of Diabetes at f-GADA testing (per 1 year increase)			0.88 (0.83, 0.94)	<0.001

Table 2: Hazard Ratios from Cox proportional regression models, stratified by t-GADA status in those f-GADA positive, (unadjusted and adjusted) for time to insulin censored at 5 years. t-GADA; truncated GAD(96-585) autoantibody. f-GADA; full length GAD(1-585) autoantibody.

Figure Legends

Figure 1: Proportions of individuals with t-GADA (A), IgG1-restricted f-GADA response (B) and high affinity f-GADA response (C). T1D; Type 1 diabetes. T2D; type 2 diabetes. t-GADA; truncated GAD(96-585) autoantibody. f-GADA; full length GAD(1-585) autoantibody.

Figure 2: Kaplan-Meier plots of probability of requiring insulin therapy during 5-year follow-up, in those clinically diagnosed with type 2 diabetes. A) Stratified by risk group of f-GADA and t-GADA positivity. Solid lines represent f-GADA positive groups and dashed lines represent f-GADA negative group. Blue line indicates t-GADA negative and red line is t-GADA positive. B) Stratified by risk group of f-GADA positivity and subclass. Solid lines represent f-GADA positive groups and dashed line represent f-GADA negative group. Blue line indicates IgG unrestricted response and red line is IgG1 restricted response. C) Stratified by risk group of f-GADA positivity and affinity. Solid lines represent f-GADA positive groups and dashed line represent f-GADA negative group. Blue line indicates a moderate/low affinity f-GADA response and red line is a high affinity f-GADA response. +, positive. -, negative.