HHS Public Access

Author manuscript

Diabet Med. Author manuscript; available in PMC 2020 November 01.

Published in final edited form as:

Diabet Med. 2019 November ; 36(11): 1375–1383. doi:10.1111/dme.13827.

Reduced display of conformational epitopes in the N-terminal truncated GAD65 isoform: relevance for people with stiff person syndrome or DQ8/8-positive Type 1 diabetes mellitus

C. S. Hampe[1], **J. R. Radtke**[1], **A. Wester**[2], **A. Carlsson**[2], **E. Cedervall**[3], **B. Jönsson**[4], **S. A. Ivarsson**[2], **H. Elding Larsson**[2], **K. Larsson**[5], **B. Lindberg**[2], **J. Neiderud**[6], **O. Rolandsson**[7], **Å. Lernmark**[2]

[1]Department of Medicine, University of Washington, Seattle, WA, USA

[2]Department of Clinical Sciences, Lund University/CRC, Skåne University Hospital SUS

[3]Department of Paediatrics, Ängelholm Hospital, Ängelholm

[4]Department of Paediatrics, Ystad Hospital, Ystad

[5]Department of Paediatrics, Kristianstad Hospital, Kristianstad

[6]Department of Paediatrics, Helsingborg Hospital, Helsingborg

[7]Department of Public Health and Clinical Medicine, Section of Family Medicine, Umeå University, Umeå, Sweden

Abstract

Aims—To investigate whether the N-terminal truncated glutamic acid decarboxylase 65 (GAD65) isoform is as well recognized by people with stiff person syndrome as it is by people with Type 1 diabetes, and whether conformational GAD65 antibody epitopes are displayed properly by the isoform.

Methods—GAD65 antibody-positive healthy individuals (n=13), people with stiff-person syndrome (n=15) and children with new-onset Type 1 diabetes (n=654) were analysed to determine binding to full-length GAD65 and the N-terminal truncated GAD65 isoform in each of these settings. GAD65 autoantibody epitope specificity was correlated with binding ratios of full-length GAD65/N-terminal truncated GAD65.

Results—The N-terminal truncated GAD65 isoform was significantly less recognized in GAD65Ab-positive people with stiff-person syndrome (P=0.002) and in healthy individuals (P=0.0001) than in people with Type 1 diabetes. Moreover, at least two specific conformational GAD65Ab epitopes were not, or were only partially, presented by the N-terminal truncated GAD65 isoform compared to full-length GAD65. Finally, an N-terminal conformational GAD65Ab epitope was significantly less recognized in DQ8/8 positive individuals with Type 1 diabetes (P=0.02).

Correspondence to: Christiane Hampe. champe@u.washington.edu.

Competing interests

None declared.

Conclusions—In people with stiff person syndrome preferred binding to the full-length GAD65 isoform over the N-terminal truncated molecule was observed. This binding characteristic is probably attributable to reduced presentation of two conformational epitopes by the N-terminal truncated molecule. These findings support the notion of disease-specific GAD65Ab epitope specificities and emphasize the need to evaluate the applicability of novel assays for different medical conditions.

Introduction

Autoantibodies directed against the 65kDa isoform of glutamate decarboxylase (GAD65Ab) are established markers for autoimmunity in autoimmune diabetes [1] and neurological disorders, including stiff person syndrome and cerebellar ataxias [2,3]. Together with other β-cell autoantibodies, GAD65Ab are valuable in the prediction of Type 1 diabetes mellitus in first-degree relatives of people with Type 1 diabetes and in the general population [4–6].

GAD65Ab detection assays rely on recombinant glutamate decarboxylase (GAD)65, expressed in different systems, including yeast, bacteria, insect cells, mammalian cells and cell-free transcription and translation systems [7–10]. These assays were evaluated for sensitivity and specificity through the Diabetes Antibody Standardization Programme and the Islet Autoantibody Standardization Programme [11]. It is important to identify GAD65 constructs with higher sensitivity and higher specificity in order to improve diagnosis and prediction; however these constructs should be characterized carefully, with evaluation of their conformation and display of disease-specific antibody epitopes. The clinical relevance of this goal is the avoidance of misdiagnoses of conditions with GAD65-associated autoimmunity.

Particular attention needs to be paid to the preservation of the antigen's conformation, because GAD65Ab in people with Type 1 diabetes recognize predominantly conformational epitopes [12–15]. A recently developed N-terminal truncated GAD65 construct showed remarkably high sensitivity and specificity compared with the full-length antigen [16–18]. These results suggest that the N-terminus may not harbour GAD65Ab epitopes relevant to Type 1 diabetes. The observation that the majority of Type 1 diabetes-associated GAD65Ab recognize epitopes located in the middle and C-terminal region of GAD65 supports this notion [12,19–21].

In a recent study, we investigated binding of GAD65Ab to truncated GAD65 and full-length GAD65 in a large cohort of children newly diagnosed with Type 1 diabetes [22]. We found that binding to full-length and truncated GAD65 correlated with comparable diagnostic specificities. Moreover, the truncated GAD65 construct appeared to allow access to an epitope that is more frequently recognized by GAD65Ab in HLA DQ8/8-positive individuals with Type 1 diabetes [22].

The display of GAD65Ab epitopes recognized by GAD65Ab present in people with stiff person syndrome by the N-terminal truncated GAD65 isoform has not been assessed. Titres of GAD65Ab in people with stiff person syndrome typically exceed those found in people with Type 1 diabetes 100–1000-fold [23] and recognize both linear and conformational

epitopes [24,25], which, to some extent, differ from those in people with Type 1 diabetes. We compared GAD65Ab recognition to both isoforms in people with stiff person syndrome, GAD65Ab-positive healthy individuals and people with Type 1 diabetes. Moreover, we correlated reactivity of both isoforms with specific GAD65Ab epitope binding using an epitope mapping assay based on competition with monoclonal GAD65Ab [12]. The major advantage of this assay is that the conformation of GAD65 is uncompromised. The assay has been successfully used to identify disease-specific GAD65Ab epitopes in people with Type 1 diabetes, in people with stiff person syndrome, in people with latent autoimmune diabetes in adults and in healthy individuals [12].

Materials and methods

Type 1 diabetes cohort

The Type 1 diabetes cohort has been described in detail previously [22]. Briefly, the cohort consisted of 654 children [median (range) age 10 (1–18) years, 352 girls] diagnosed with Type 1 diabetes in 1996–2005 according to American Diabetes Association guidelines [26].

Stiff person syndrome cohort

Serum samples were obtained from GAD65Ab-positive people with stiff person syndrome [n=15; mean (range) age 50 (29–71) years, 10 women]. These individuals were diagnosed between 1999 and 2011 at the University of Washington, Seattle, USA and Skåne University Hospital, Sweden.

Healthy individuals

GAD65Ab-positive healthy individuals [n=13; mean (range) age 50 (30–60) years, eight women] were identified in a population-based screening of 2157 Swedish adults [27]. None of these individuals developed Type 1 diabetes <8 years after the samples were taken [28].

All participants in this study gave informed consent. Local institutional ethics committee approval was obtained before collection of all serum samples.

Monoclonal GAD65Ab and expression of recombinant antibody fragments

Monoclonal GAD65Ab DPA and DPD were isolated from a person with Type 1 diabetes [29] and recognized epitopes at amino acid residues 483–585 and 96–173, respectively [13]; b96.11 and b78 were isolated from a person with APS-1 and recognized epitopes at the PLP domain (amino acid residues 308–365) and the C-terminus (amino acid residues 518–540), respectively [13,30,31]. All monoclonal antibodies recognized GAD65 in only its native conformation. Human monoclonal antibody HAA1 (ATCC Manassas VA, USA, ATCC number: HB-8534) is directed against blood group A antigen and served as a control.

Gene fragments encoding antibody fragments (FAb) of the above monoclonal antibodies were cloned into the expression vector pAK19 [32], as described earlier [12]. Recombinant FAb (rFAb) were expressed in *Escherichia coli* 25F2 cells and isolated from the bacteria as described previously [12].

GAD65Ab radioligand binding assay

GAD65Ab binding to the GAD2 full-length cDNA gene and the N-truncated isoform (amino acids 96–585) was determined by radioligand binding assay in our previous study [22]. The cut-off for positivity was determined as 50U/ml for both constructs, as described previously [22].

GAD65Ab epitope mapping assay

The capacity of GAD65-specific rFAb to inhibit GAD65 binding by human serum GAD65Abs was tested in a competitive radioligand binding assay, as previously described [12]. Briefly, serum samples were incubated with radiolabelled full-length GAD65 and rFAb derived from the above monoclonal GAD65Ab. rFAb were added at the maximal concentration, as determined in competition assays using the intact monoclonal antibody as a competitor. Binding of GAD65Ab to GAD65 in the presence of rFAb was expressed as follows: counts per min of [S^{35}]GAD65 bound in the presence of rFAb/counts per min of [S^{35}]GAD65 bound in the absence of rFAb × 100. Samples that exceeded 1000 U/ml were diluted to half maximal binding capacity. The cut-off for specific competition was >15%, as determined by control rFAb HAA1. In a few cases, the rFAb-competed sample resulted in higher counts per min than the non-competed sample; this was attributable to intra-assay variations.

Statistical analysis

All samples were analysed in triplicate determinations and the intra-assay average coefficient of variation was 7%, with the highest value 20 and the lowest 0.1. The significance of differences in competition between different serum groups was tested using the non-parametric Mann–Whitney *U*-test. The significance of differences in binding to full-length and truncated GAD65 within the same group was tested with the paired Wilcoxon signed-rank test. The significance of correlation was analysed using Spearman's rank correlation test. Significance of differences in frequency was tested using Fisher exact probability test. All statistical testing was two-sided, and *P* values <0.05 were taken to indicate statistical significance. Statistical analyses were performed using the PRISM program (GraphPad, San Diego, CA, USA) and STATA (StataCorp LLC, College Station, TX, USA)

Results

Binding to full-length GAD65 vs truncated GAD65 in healthy individuals and people with stiff person syndrome.

To evaluate the relative binding to both GAD65 constructs, we analysed sera obtained from GAD65Ab-positive healthy individuals (n=13) and people with stiff person syndrome (n=15; Fig. 1).

In people with stiff person syndrome the full-length construct was bound considerably better as compared to the N-terminal truncated isoform (median GAD65Ab index of 4058 vs 2718 U/ml, respectively; P=0.0004). In healthy individuals the full-length isoform was recognized significantly better than the N-terminal truncated molecule (median GAD65Ab index of 141

vs 70 U/ml, respectively; *P*=0.0002). The GAD65Ab-binding specificity of these sera had been previously confirmed in displacement assays using recombinant GAD65 [27].

Binding to full-length GAD65 compared to N-terminal truncated GAD65 in people with Type 1 diabetes

Type 1 diabetes sera (*n*=654) were analysed for binding to the truncated and the full-length GAD65 constructs. To express preferred binding of Type 1 diabetes sera to one GAD65 construct over the other, we calculated the binding ratios of full-length GAD65/N-terminal truncated GAD65 (Fig. 1). To avoid the confounding effect of background signals, we only considered GAD65Ab-positive samples (GAD65Ab level of ≥50 U/ml for either full-length or truncated GAD65, or both; *n*=475) in the present analysis.

Fifty-eight percent (279/475) of sera showed a binding ratio of <1.0 [median (range) full-length GAD65: 179 (7–1711) U/ml, median range truncated GAD65: 220 (50–1752) U/ml] for full-length GAD65/N-terminal truncated GAD65, while the remaining 42% (196/654) of sera showed a binding ratio of >1.0 [median (range) full-length GAD65: 849 (54–2682) U/ml, median (range) truncated GAD65: 661 (38–1831) U/ml] for full-length GAD65/N-terminal truncated GAD65. We found no correlation between age at onset and binding ratio of full-length GAD65/N-terminal truncated GAD65 (data not shown). Notably, the binding ratio of full-length GAD65/N-terminal truncated GAD65 in people with Type 1 diabetes (median 1.0) was significantly lower than that in people with stiff person syndrome (median 1.3; *P*=0.002) or in healthy individuals (median 2.0; *P*=0.0001).

GAD65Ab epitope mapping

We analysed all serum samples from people with stiff person syndrome and serum samples from people with Type 1 diabetes with a GAD65Ab titre (full-length) ≥100U/ml (*n*=349) and sufficient sample volume for epitope binding using rFAb derived from human monoclonal GAD65Ab DPA, b96.11, DPD and b78, respectively (Fig. 2). Samples with GAD65Ab titres <100U/ml were excluded because the relatively high background in these samples interfered with the reliability of the competition results. Samples that exceeded 1000 U/ml were diluted to their half-maximal binding capacity.

The b96.11-defined epitope was recognized in 94% (14/15) of people with stiff person syndrome and 84% (293/349) of people with Type 1 diabetes. The DPD-defined epitope was bound in 94% (14/15) of people with stiff person syndrome and in 53% of people with Type 1 diabetes, the DPA-defined epitope was recognized in 74% (11/15) of people with stiff person syndrome and 20% of people with Type 1 diabetes, and the b78-defined epitope was recognized in 80% (12/15) of people with stiff person syndrome and 20% of people with Type 1 diabetes.

Correlations between GAD65Ab titre and epitope specificity

To determine possible correlations between GAD65Ab titres and epitope specificity we generated scatter plots for the individual rFAb (Fig. 3). Binding to the b78-defined epitope was inversely correlated with GAD65Ab titres (*P*=0.0004), while no other correlation between epitope binding and GAD65Ab titre was observed.

Associations between GAD65Ab epitope specificity and full-length/truncated GAD65Ab titre ratio

To investigate the presentation of GAD65Ab epitope regions on full-length and N-terminal truncated GAD65, we analysed binding of the four human monoclonal antibodies to full-length and N-terminal truncated GAD65 (data not shown). While DPA, b96.11 and b78 bound equally well to either isoform, DPD only recognized the full-length construct and showed no detectable binding to the N-terminal truncated isoform (Fig. S1).

To further investigate the correlations between GAD65Ab epitope specificities and the full-length/truncated GAD65Ab titre ratio, we plotted binding results of Type 1 diabetes samples showing competition with the respective rFAb (DPA, n=71; b96.11, n=293; DPD, n=184; b78, n=73; binding ≤85%) against the full-length/truncated GAD65Ab ratio.

Binding to the b78- and the DPD-defined epitopes correlated directly with the ratio of full-length/truncated GAD65Ab titre (Parson r: −0.36, P=0.001, Parson r: −0.16; P=0.02, respectively [Fig. 4]), indicating that GAD65Ab recognizing the b78- and DPD-defined epitopes preferred binding to the full-length GAD65 construct. No other correlations between specific epitopes and the full-length/truncated GAD65Ab titre ratio were observed. Moreover, the number of recognized epitope by the samples did not correlate with the full-length/truncated GAD65Ab titre ratio.

Associations between GAD65Ab epitope specificities, autoantibody specificities and HLA class II genotypes

The frequency of binding specificities in HLA-class II genotypes HLA-DQ 2/8, 8/8,8/X, 2/2, 2/X and X/X was determined for individuals with GAD65Ab levels ≤100 U/ml and completed rFAb testing. We found that DQ8/8-positive children had a lower frequency of GAD65Ab recognizing the DPD-defined epitope: the DPD-defined epitope was recognized in 35% (16/45) of DQ8/8-positive children compared with 55% (167/304) of DQ8/8-negative children (P=0.02).

No other associations between the tested epitopes and HLA genotypes or age were noted.

Discussion

In the present study we analysed GAD65Ab epitope specificities in the sera of people with stiff person syndrome, in GAD65Ab-positive healthy individuals and in people with Type 1 diabetes to investigate whether their respective GAD65Ab epitope pattern affected binding to full-length vs truncated GAD65 isoforms.

In people with stiff person syndrome and healthy individuals, a significant preference of binding to the full-length GAD65 molecule was observed, while the majority of Type 1 diabetes sera (66%) showed higher binding to N-terminal truncated GAD65. While the findings in healthy individuals and people with Type 1 diabetes mainly confirmed earlier findings [16–18,22], the binding preference of full-length over N-terminal truncated GAD65 by GAD65Ab in people with stiff person syndrome had not been assessed previously.

GAD65Ab can be detected in the sera of 80% of people newly diagnosed with Type 1 diabetes [33], 5–10% of people with T2DM (classified as latent autoimmune diabetes in adults) [34,35], 60% of people with stiff person syndrome [36], and 2–3% of healthy individuals [37]. However, GAD65Ab in these clinical phenotypes differ significantly in titre and epitope specificities [12,14,19,20,25,38]. The majority of Type 1 diabetes-associated GAD65Ab epitopes are conformational and are located at the middle and C-terminal region of the GAD65 molecule, while sera from people with stiff person syndrome recognize both conformational and linear epitopes located across the entire molecule [24,25].

To determine the cause of these differences in binding preference, we investigated the association of individual GAD65Ab epitopes with the ratio of full-length GAD65Ab titre over truncated GAD65Ab titre.

Consistent with our previous studies [25,39], we found significant recognition of GAD65Ab epitopes defined by b78 and DPD in people with stiff person syndrome. Moreover, people with Type 1 diabetes with preferential binding to the full-length construct showed higher frequencies of GAD65Ab recognizing the DPD- and b78-defined epitopes. These results suggest that the DPD- and b78-defined epitopes are not fully displayed by the truncated GAD65 isoform. Indeed, monoclonal antibody DPD failed to recognize the truncated GAD65 construct, although its epitope region (amino acids 96–173) is downstream of the deleted N-terminus (amino acids 1–96), suggesting that the N-terminal amino acids affect epitopes contained in the amino acids adjacent to the deleted protein portion. We cannot exclude the possibility that some of the differences in epitope pattern between people with Type 1 diabetes and those with stiff person syndrome may be attributable to the characteristically lower GAD65Ab titres in people with Type 1 diabetes. Indeed, a previous report showed that Type 1 diabetes sera with high GAD65Ab titres had antibody patterns similar to those observed in people with stiff person syndrome [40]. Our finding that binding to the b78-defined epitope was significantly better in high GAD65Ab sera from people with Type 1 diabetes confirms these earlier findings, and may in part explain the differences in recognition of the b78-defined epitope between people with Type 1 diabetes and people with stiff person syndrome. Clinical information regarding possible neurological symptoms was not available for these participants; however, no correlation between GAD65Ab titre and the DPD-defined epitope was observed, suggesting that these differences were disease-specific.

Recognition of the DPD-defined GAD65Ab epitope may also explain our previous finding that the truncated GAD65 construct was recognized significantly better in DQ8/8-positive children [22], as lower recognition of the DPD-defined epitope was observed in DQ8/8-positive children.

A limitation of the present study was the relatively small number of samples in the group with stiff person syndrome and in GAD65Ab-positive healthy controls and the differences in ages between the different cohorts. Age at onset did not correlate, however, with binding ratio of full-length GAD65/N-terminal truncated GAD65 or recognition of any of the epitopes, so that it is unlikely that the observed differences in binding pattern were affected by the participants' ages.

In conclusion, our data show that in people with stiff person syndrome and in healthy individuals there was significantly higher binding to the full-length molecule, while in people with Type 1 diabetes the truncated isoform was preferred. These data demonstrate the importance of carefully evaluating novel antigenic constructs for their binding capacities across different clinical phenotypes. The present data also suggest that the N-terminus harbours GAD65Ab epitopes that are particularly relevant for binding of GAD65Ab present in healthy individuals and people with stiff person syndrome, but are also bound in ~50% of people with Type 1 diabetes. Studies into possible associations with β-cell function or other clinical measurements are needed to determine whether the specificity and sensitivity of the truncated GAD65 construct is comparable across different clinical subtypes of people with Type 1 diabetes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors thank Ida Jönsson (Lund University Clinical Research Centre, Skåne University Hospital, Malmö, Sweden) for technical assistance.

Funding sources

A.W. was supported by a summer student stipend from the Lund University Medical Faculty Foundation. This work was also supported by the Swedish Research Council, the Swedish Diabetes Foundation, the Skåne University Hospital Fund, the Skåne County Council for Research and Development, and the Swedish Child Diabetes Foundation, and the National Institutes of Health (DK26190 to C.S.H.)

References

1. Uibo R, Lernmark A. GAD65 autoimmunity-clinical studies. Adv Immunol 2008; 100:39–78. [PubMed: 19111163]
2. Ali F, Rowley M, Jayakrishnan B, Teuber S, Gershwin ME, Mackay IR. Stiff-person syndrome (SPS) and anti-GAD-related CNS degenerations: protean additions to the autoimmune central neuropathies. J Autoimmun 2011; 37:79–87. [PubMed: 21680149]
3. Manto MU, Hampe CS, Rogemond V, Honnorat J. Respective implications of glutamate decarboxylase antibodies in stiff person syndrome and cerebellar ataxia. Orphanet J Rare Dis 2011; 6:3. [PubMed: 21294897]
4. Verge CF, Gianani R, Kawasaki E, Yu L, Pietropaolo M, Chase HP et al. Number of autoantibodies (against insulin, GAD or ICA512/IA2) rather than particular autoantibody specificities determines risk of type I diabetes. J Autoimmun 1996; 9:379–383. [PubMed: 8816974]
5. Bingley PJ, Bonifacio E, Williams AJK, Genovese S, Bottazzo GF, Gale EAM. Prediction of IDDM in the general population: Strategies based on combinations of autoantibody markers. Diabetes 1997; 46:1701–1710. [PubMed: 9356015]
6. Krischer JP, Lynch KF, Schatz DA, Ilonen J, Lernmark A, Hagopian WA et al. The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. Diabetologia 2015; 58:980–987. [PubMed: 25660258]
7. Papouchado ML, Valdez SN, Ghiringhelli D, Poskus E, Ermácora MR. Expression of properly folded human glutamate decarboxylase 65 as a fusion protein in Escherichia coli. Eur J Biochem 1997; 246:350–359. [PubMed: 9208924]
8. Moody AJ, Hejnaes KR, Marshall MO, Larsen FS, Boel E, Svendsen I et al. Isolation by anion-exchange of immunologically and enzymatically active human islet glutamic acid decarboxylase 65 overexpressed in Sf9 insect cells. Diabetologia 1995; 38:14–23. [PubMed: 7744224]

9. Powell M, Prentice L, Asawa T, Kato R, Sawicka J, Tanaka H et al. Glutamic acid decarboxylase autoantibody assay using I-125-labelled recombinant GAD(65) produced in yeast. Clinica Chimica Acta 1996; 256:175–188.
10. Grubin CE, Daniels T, Toivola B, Landin-Olsson M, Hagopian WA, Li L et al. A novel radioligand binding assay to determine diagnostic accuracy of isoform-specific glutamic acid decarboxylase antibodies in childhood IDDM. Diabetologia 1994; 37:344–350. [PubMed: 8063033]
11. Bingley PJ, Bonifacio E, Mueller PW. Diabetes Antibody Standardization Program: first assay proficiency evaluation. Diabetes 2003; 52:1128–1136. [PubMed: 12716742]
12. Padoa CJ, Banga JP, Madec AM, Ziegler M, Schlosser M, Ortqvist E et al. Recombinant Fabs of human monoclonal antibodies specific to the middle epitope of GAD65 inhibit type 1 diabetes-specific GAD65Abs. Diabetes 2003; 52:2689–2695. [PubMed: 14578287]
13. Schwartz HL, Chandonia JM, Kash SF, Kanaani J, Tunnell E, Domingo A et al. High-resolution autoreactive epitope mapping and structural modeling of the 65 kDa form of human glutamic acid decarboxylase. J Mol Biol 1999; 287:983–999. [PubMed: 10222205]
14. Ujihara N, Daw K, Gianani R, Boel E, Yu L, Powers AC. Identification of glutamic acid decarboxylase autoantibody heterogeneity and epitope regions in type I diabetes. Diabetes 1994; 43:968–975. [PubMed: 8039604]
15. Tuomi T, Rowley MJ, Knowles WJ, Chen QY, McAnally T, Zimmet PZ et al. Autoantigenic properties of native and denatured glutamic acid decarboxylase: evidence for a conformational epitope. Clin Immunol Immunopathol 1994; 71:53–59. [PubMed: 7511084]
16. Williams AJ, Lampasona V, Wyatt R, Brigatti C, Gillespie KM, Bingley PJ et al. Reactivity to N-Terminally Truncated GAD65(96–585) Identifies GAD Autoantibodies That Are More Closely Associated With Diabetes Progression in Relatives of Patients With Type 1 Diabetes. Diabetes 2015; 64:3247–3252. [PubMed: 26001397]
17. Williams AJ, Lampasona V, Schlosser M, Mueller PW, Pittman DL, Winter WE et al. Detection of Antibodies Directed to the N-Terminal Region of GAD Is Dependent on Assay Format and Contributes to Differences in the Specificity of GAD Autoantibody Assays for Type 1 Diabetes. Diabetes 2015; 64:3239–3246. [PubMed: 25972570]
18. Giannopoulou EZ, Winkler C, Chmiel R, Matzke C, Scholz M, Beyerlein A et al. Islet autoantibody phenotypes and incidence in children at increased risk for type 1 diabetes. Diabetologia 2015; 58:2317–2323. [PubMed: 26138334]
19. Hampe CS, Hammerle LP, Bekris L, Ortqvist E, Kockum I, Rolandsson O et al. Recognition of Glutamic Acid Decarboxylase (GAD) by Autoantibodies from Different GAD Antibody-Positive Phenotypes. J Clin Endocrinol Metab 2000; 85:4671–4679. [PubMed: 11134126]
20. Powers AC, Bavik K, Tremble J, Daw K, Scherbaum WA, Banga JP. Comparative analysis of epitope recognition of glutamic acid decarboxylase (GAD) by autoantibodies from different autoimmune disorders. Clin Exp Immunol 1999; 118:349–356. [PubMed: 10594551]
21. Syren K, Lindsay L, Stoehrer B, Jury K, Luhder F, Baekkeskov S et al. Immune reactivity of diabetes-associated human monoclonal autoantibodies defines multiple epitopes and detects two domain boundaries in glutamate decarboxylase. J Immunol 1996; 157:5208–5214. [PubMed: 8943434]
22. Wester A, Skärstrand H, Lind A, Ramelius A, Carlsson A, Cedervall E et al. An Increased Diagnostic Sensitivity of Truncated GAD65 Autoantibodies in Type 1 Diabetes May Be Related to HLA-DQ8. Diabetes 2017; 66:735–740. [PubMed: 28028075]
23. Daw K, Powers AC. Two distinct glutamic acid decarboxylase auto-antibody specificities in IDDM target difference epitopes. Diabetes 1995; 44:216–220. [PubMed: 7532143]
24. Bjork E, Velloso LA, Kampe O, Karlsson FA. GAD autoantibodies in IDDM, stiff-man syndrome, and autoimmune polyendocrine syndrome type I recognize different epitopes. Diabetes 1994; 43:161–165. [PubMed: 7505244]
25. Raju R, Foote J, Banga JP, Hall TR, Padoa CJ, Dalakas MC et al. Analysis of GAD65 autoantibodies in Stiff-Person syndrome patients. J Immunol 2005; 175:7755–7762. [PubMed: 16301686]
26. Standards of medical care in diabetes–2010. Diabetes Care 2010; 33 (Suppl. 1):S11–61. [PubMed: 20042772]

27. Rolandsson O, Hagg E, Hampe C, Sullivan EP Jr, Nilsson M, Jansson G et al. Glutamate decarboxylase (GAD65) and tyrosine phosphatase-like protein (IA-2) autoantibodies index in a regional population is related to glucose intolerance and body mass index. Diabetologia 1999; 42:555–559. [PubMed: 10333047]
28. Rolandsson O, Hagg E, Nilsson M, Hallmans G, Mincheva-Nilsson L, Lernmark A. Prediction of diabetes with body mass index, oral glucose tolerance test and islet cell autoantibodies in a regional population. J Intern Med 2001; 249:279–288. [PubMed: 11298847]
29. Madec AM, Rousset F, Ho S, Robert F, Thivolet C, Orgiazzi J et al. Four IgG anti-islet human monoclonal antibodies isolated from a type 1 diabetes patient recognize distinct epitopes of glutamic acid decarboxylase 65 and are somatically mutated. J Immunol 1996; 156:3541–3549. [PubMed: 8617984]
30. Tremble J, Morgenthaler NG, Vlug A, Powers AC, Christie MR, Scherbaum WA et al. Human B cells secreting immunoglobulin G to glutamic acid decarboxylase- 65 from a nondiabetic patient with multiple autoantibodies and Graves' disease: a comparison with those present in type 1 diabetes. J Clin Endocrinol Metab 1997; 82:2664–2670. [PubMed: 9253351]
31. Fenalti G, Hampe CS, Arafat Y, Law RH, Banga JP, Mackay IR et al. C-terminal clustering of autoantibody and T cell determinants on the structure of GAD65 provide insights into the molecular basis of autoreactivity. Diabetes 2008; 57:1293–1301. [PubMed: 18184926]
32. Carter P, Kelley RF, Rodrigues ML, Snedecor B, Covarrubias M, Velligan MD et al. High level Escherichia coli expression and production of a bivalent humanized antibody fragment. Biotechnology (N Y) 1992; 10:163–167. [PubMed: 1368228]
33. Baekkeskov S, Aanstoot HJ, Christgau S, Reetz A, Solimena M, Cascalho M et al. Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature 1990; 347:151–156. [PubMed: 1697648]
34. Hawa MI, Kolb H, Schloot N, Beyan H, Paschou SA, Buzzetti R et al. Adult-onset autoimmune diabetes in Europe is prevalent with a broad clinical phenotype: Action LADA 7. Diabetes Care 2013; 36:908–913. [PubMed: 23248199]
35. Turner R, Stratton I, Horton V, Manley S, Zimmet P, Mackay IR et al. UKPDS 25: autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. UK Prospective Diabetes Study Group [published erratum appears in Lancet 1998 Jan 31;351(9099):376]. Lancet 1997; 350:1288–1293. [PubMed: 9357409]
36. Solimena M, Folli F, Aparisi R, Pozza G, De Camilli P. Autoantibodies to GABA-ergic neurons and pancreatic beta cells in stiff-man syndrome. N Engl J Med 1990; 322:1555–1560. [PubMed: 2135382]
37. Rolandsson O, Hampe CS, Wennberg P, Radtke J, Langenberg C, Wareham N, Group E-IS. Prevalence and Regional Distribution of Autoantibodies Against GAD65Ab in a European Population Without Diabetes: The EPIC-InterAct Study. Diabetes Care 2015; 38:e114–115. [PubMed: 26207060]
38. Daw K, Ujihara N, Atkinson M, Powers AC. Glutamic acid decarboxylase autoantibodies in stiff-man syndrome and insulin-dependent diabetes mellitus exhibit similarities and differences in epitope recognition. J Immunol 1996; 156:818–825. [PubMed: 8543838]
39. Manto M, Honnorat J, Hampe CS, Guerra-Narbona R, Lopez-Ramos JC, Delgado-Garcia JM et al. Disease-specific monoclonal antibodies targeting glutamate decarboxylase impair GABAergic neurotransmission and affect motor learning and behavioral functions. Front Behav Neurosci 2015; 9:78. [PubMed: 25870548]
40. Piquer S, Belloni C, Lampasona V, Bazzigaluppi E, Vianello M, Giometto B et al. Humoral autoimmune responses to glutamic acid decarboxylase have similar target epitopes and subclass that show titer-dependent disease association. Clin Immunol 2005; 117:31–35. [PubMed: 16027042]

What's new?

- Amino acid residues 1–95 of glutamic acid decarboxylase (GAD) 65 have little impact on GAD65 binding by the majority of Type 1 diabetes mellitus-associated GAD65 antibodies.
- N-terminal truncated GAD65(96–585) shows reduced display of autoantibody epitopes relevant to people with stiff person syndrome or DQ8/8-positive Type 1 diabetes mellitus.
- High-titre GAD65 antibody sera from people with Type 1 diabetes mellitus share some antibody specificities with GAD65 antibodies from people with stiff person syndrome.
- Specific GAD65 autoantibody epitope recognition may aid in the identification of people with stiff person syndrome, especially those with only moderate GAD65 antibody levels.

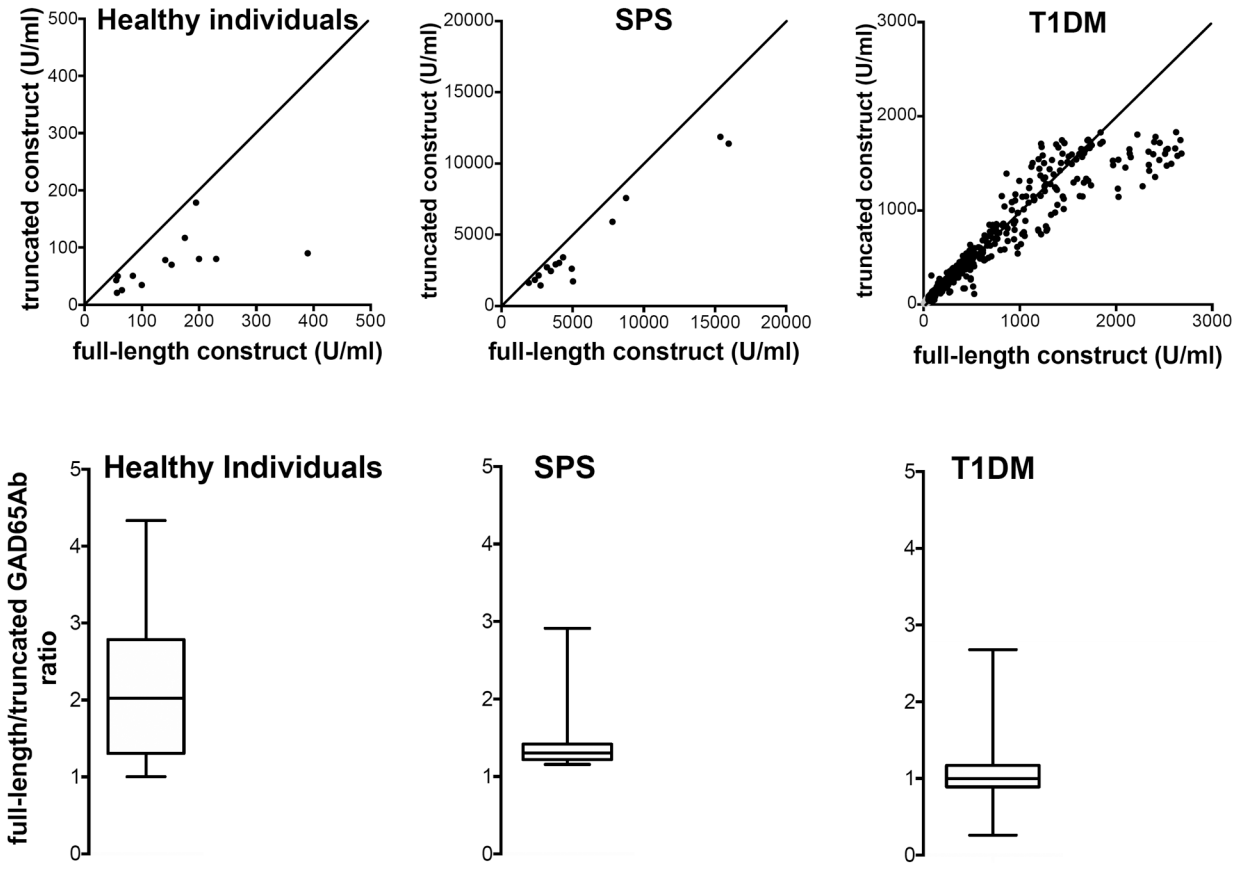

FIGURE 1.

Glutamic acid decarboxylase (GAD)65 autoantibody (Ab) binding to full-length and truncated GAD65. Binding to full-length GAD65 is plotted against N-terminal truncated GAD65 for people with stiff person syndrome, healthy individuals and people with Type 1 diabetes is shown in the respective upper panels. A line of equivalence is shown for each plot. The ratios of full-length GAD65/N-terminal truncated GAD65 are shown in the respective lower panels.

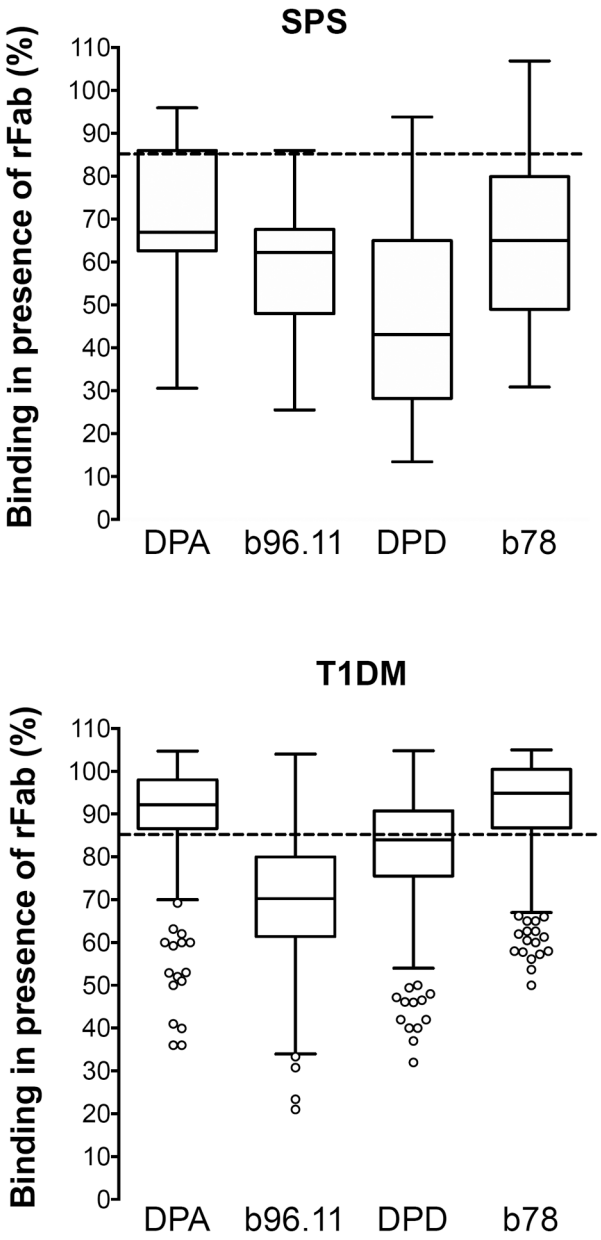

FIGURE 2.

Glutamic acid decarboxylase (GAD)65 autoantibody (Ab) epitope specificity. Binding of GAD65Ab in people with stiff person syndrome (upper panel) and Type 1 diabetes (lower panel) in the presence of recombinant antibody fragments (rFAb) derived from monoclonal GAD65Ab DPA, b96.11, DPD and b78 was determined. Binding in the absence of rFAb was set to 100%. Data are presented as box and whisker plots with Tukey-style whiskers. The cut-off for significant competition (85%) is indicated by the dotted line.

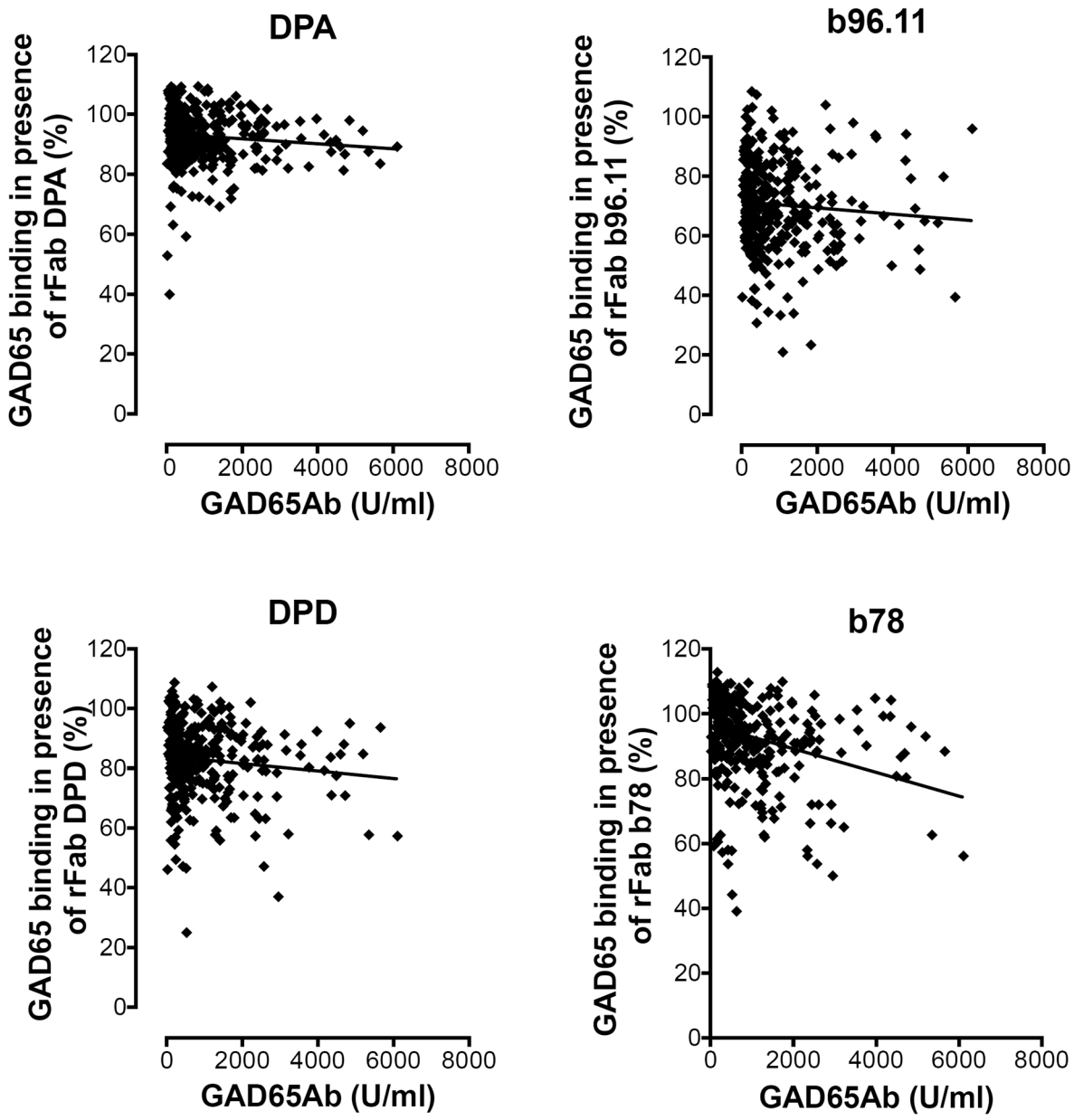

FIGURE 3.

Correlation of epitope binding with full-length glutamic acid decarboxylase (GAD)65 autoantibody (Ab) titre. Binding of serum samples from individuals with stiff person syndrome and individuals with Type 1 diabetes with GAD65Ab titre (full-length) ≥100U/ml (Type 1 diabetes: n=349) to GAD65 in the presence of recombinant antibody fragment (rFAb) DPA, b96.11, b78 and DPD is plotted against full-length GAD65Ab titre. Linear regression lines are shown.

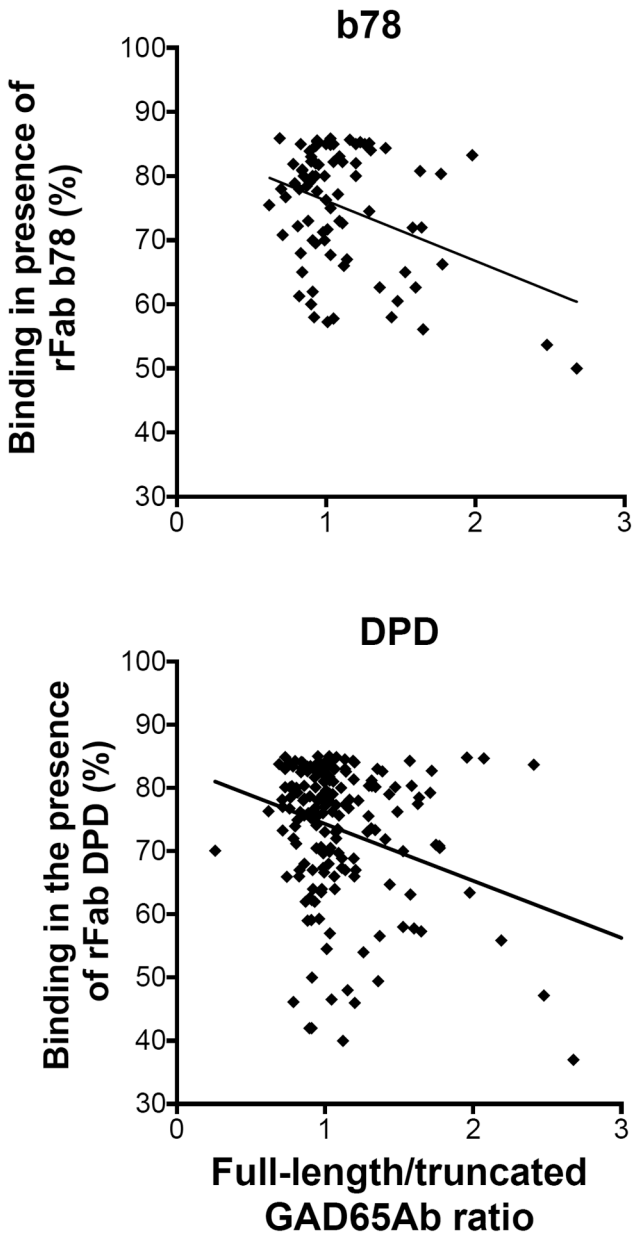

FIGURE 4.

Correlation of b78- and DPD-defined epitopes with full-length/truncated glutamic acid decarboxylase (GAD)65 autoantibody (Ab) titre ratio. Competition of GAD65 binding by recombinant antibody fragment (rFAb) b78 or DPD of Type 1 diabetes samples whose binding was competed by at least 15% (n=73 and 184, respectively) is plotted against full-length/truncated GAD65Ab titre ratio. Linear regression lines are shown. The y-axis is truncated at 30% to allow higher resolution of the data.