Clinical and genetic characteristics of people with type 1 diabetes who have discrepancies in titers of anti-glutamic acid decarboxylase antibody measured by radioimmunoassay and enzyme-linked immunosorbent assay

Satoshi Takagi1, Junnosuke Miura1*, Sari Hoshina1, Yasuko Uchigata1,2, Tetsuya Babazono1

1Diabetes Center, Tokyo Women’s Medical University School of Medicine, and 2Tokyo Women’s Medical University Medical Center East, Tokyo, Japan

Keywords
Glutamic acid decarboxylase, Human leukocyte antigens, Type 1 diabetes

*Correspondence
Junnosuke Miura
Tel.: +81-3-3353-8111
Fax: +81-3-3358-1941
E-mail address: jmiura.dmc@twmu.ac.jp

J Diabetes Investig 2020; 11: 356–362
doi: 10.1111/jdi.13111

ABSTRACT
Aims/Introduction: The aim of the present study was to compare the clinical and genetic characteristics between people with type 1 diabetes who were positive and negative for autoantibodies against glutamic acid decarboxylase (GADA) measured by enzyme-linked immunosorbent assay (ELISA) with low-titer GADA measured by radioimmunoassay.

Materials and Methods: Among Japanese people with type 1 diabetes in whom GADA were measured by both ELISA and radioimmunoassay, those who had low titers of GADA measured by radioimmunoassay (1.5–10 U/mL), regardless of positivity for GADA measured by ELISA, were studied. There were 65 participants with acute-onset type 1 diabetes and 30 participants with slowly progressive insulin-dependent diabetes mellitus. Clinical characteristics and human leukocyte antigen types were compared in ELISA-positive (≥5 U/mL) and ELISA-negative participants. Endogenous insulin secretion was evaluated by C-peptide index.

Results: Among participants with slowly progressive insulin-dependent diabetes mellitus, postprandial C-peptide index was significantly higher in ELISA-negative participants than in ELISA-positive participants (r = 0.619, P = 0.002). Among 52 participants whose human leukocyte antigen typing was carried out, all of the participants with slowly progressive insulin-dependent diabetes mellitus who had DRB1*09:01 were positive by GADA-ELISA (P = 0.021). In acute-onset type 1 diabetes participants, there were no significant differences for the C-peptide index and human leukocyte antigen genotypes.

Conclusions: The difference in the positivity for GADA-ELISA might reflect cytotoxicity toward pancreatic β-cells and preservation of endogenous insulin secretion in people with slowly progressive insulin-dependent diabetes mellitus. We also suggest that the difference in the GADA-ELISA-specific epitope depends on the human leukocyte antigen genotype.

INTRODUCTION
Type 1 diabetes is mainly caused by autoimmune destruction of pancreatic β-cells. Current autoimmune markers for immune-mediated diabetes include autoantibodies against glutamic acid decarboxylase (GADA), insulinoma-associated antigen-2 and zinc transporter-8. Among these, GADA appears most frequently at the onset of type 1 diabetes, and 71% of newly diagnosed Japanese people with type 1 diabetes have GADA. Therefore, measurement of GADA is vital for diagnosing immune-mediated diabetes.

In Japan, until January 2016, GADA had been measured using radioimmunoassay (RIA; RSR Limited, Cardiff, UK). From January 2016, the method was exclusively replaced by
enzyme-linked immunosorbent assay (ELISA; RSR Limited). The results of the two measurement methods diverge by 9.8% in people with acute-onset type 1 diabetes mellitus or fulminant type 1 diabetes mellitus, and by 25.4% in people with slowly progressive insulin-dependent diabetes mellitus (SPIDDM)\(^3\). These differences are more prominent in people with lower positive titers of \(<10.0\) U/mL measured by GADA-RIA\(^4\). There are few reports comparing the clinical features of people who were positive versus people who were negative for GADA-ELISA among those positive for GADA-RIA. Oikawa \textit{et al.}\(^3\) showed that the serum C-peptide levels of GADA-ELISA-positive people were significantly lower than those of GADA-ELISA-negative people. However, the differences in sample collection dates represented a limitation of the report. In other words, the blood samples for GADA-RIA and GADA-ELISA were taken on separate days, possibly several years apart. Therefore, the possibility that the antibody titer decreased naturally during the storage cannot be ruled out. In addition, the association between GADA-ELISA positivity and human leukocyte antigen (HLA) was not studied in the report. Therefore, we carried out the present study to investigate the clinical features, pathophysiology, and characteristics of HLA in people with type 1 diabetes who are low-titer-positive for GADA-RIA, according to the positivity for GADA by ELISA in samples collected on the same day.

**METHODS**

**Study population**

This was a single-center, cross-sectional study, consisting of Japanese people with type 1 diabetes, recruited from the Diabetes Center of Tokyo Women’s Medical University School of Medicine. Those for whom GADA-RIA measured from 2013 to 2015 was 1.5–10 U/mL, indicating low-positive titers, were included. Participants aged \(<20\) years, those who had undergone pancreas transplantation, those diagnosed with fulminant type 1 diabetes\(^5\), and those with an unclear diabetes classification were excluded.

**Methods**

Serum samples obtained at the measurement of GADA with RIA from 2013 to 2015 were frozen and used for the measurement of GADA by ELISA in 2016. The GADAb “Cosmic” (RSR Limited, Cardiff, UK; marketing authorization holder: Cosmic Corporation Co., Ltd, Tokyo, Japan) and GADAb ELISA “Cosmic” kits (RSR Limited; Marketing authorization holder: Cosmic Corporation Co., Ltd) were used for GADA-RIA and GADA-ELISA, respectively. Samples were measured by SRL Inc. (Tokyo, Japan). The cut-off value of GADA-RIA was 1.5 U/mL, and that of GADA-ELISA was 5.0 U/mL.

Clinical, anthropometric and laboratory data, including age, sex and medical history (including duration of diabetes and chronic complications of diabetes, insulin and oral hypoglycemic agent dosages, height, bodyweight, glycated hemoglobin, HLA [class I: A, B; class II: DRB1, DQB1], serum C-peptide, plasma glucose [fasting and/or postprandial], estimated glomerular filtration rate, insulinoma-associated antigen-2, and insulin autoantibody) were extracted from the medical records of Tokyo Women’s Medical University School of Medicine, Tokyo, Japan. Body mass index was calculated as body weight (kg) divided by the square of height (m). Postprandial C-peptide index (p-CPI), a measure of insulin secretion, was calculated as serum postprandial C-peptide (p-CPR) / postprandial plasma glucose \(\times 100\) (ng/mg). For comparison of p-CPR and p-CPI, participants whose estimated glomerular filtration rate was \(<30\) ml/min/1.73 m\(^2\) were excluded, as serum C-peptide is falsely elevated in these participants\(^6\). Insulin dose was expressed by dividing by body weight (units/kg).

Participants were divided into those with acute-onset type 1 diabetes mellitus and slowly progressive insulin-dependent diabetes mellitus, and into GADA-ELISA-positive and GADA-ELISA-negative groups. The diagnoses of acute-onset type 1 diabetes mellitus and slowly progressive insulin-dependent diabetes mellitus were based on the criteria of the Japan Diabetes Society\(^7,8\). Briefly, acute-onset type 1 diabetes mellitus is characterized by diabetic ketosis or ketoacidosis within 3 months of onset of hyperglycemic symptoms, and requirement of continuous insulin therapy\(^7\). Slowly progressive insulin-dependent diabetes mellitus is characterized by positive GADA and/or islet cell antibodies during the disease course and insulin is not necessary immediately after diagnosis\(^8\). The protocol for this research project has been approved by a suitably constituted ethics committee of the institution (Committee of Tokyo Women’s Medical University, approval number 4151-R and 367.), and it conforms to the provisions of the Declaration of Helsinki.

**Statistical analysis**

Continuous variables were analyzed by the \(t\)-test, Mann–Whitney \(U\)-test and analysis of variance. Categorical variables were analyzed by the Fisher’s exact probability test and Mann–Whitney \(U\)-test. A \(P\)-value \(<0.05\) was regarded as significant. Statistical analysis was carried out using JMP version 13.0 (SAS Institute Inc., Cary, NC, USA).

**RESULTS**

**Clinical characteristics of participants by GADA-ELISA titer**

A total of 95 participants were involved in the present study. The clinical features of 74 GADA-ELISA-positive and 21 GADA-ELISA-negative participants are shown in Table 1. There was a significant positive correlation between GADA-ELISA and GADA-RIA in participants with acute-onset type 1 diabetes mellitus \((P < 0.0001)\), but not in participants with slowly progressive insulin-dependent diabetes mellitus \((P = 0.0721);\) Spearman’s rank correlation coefficient (Figure 1).

**Comparison according to diabetes classification**

In participants with acute-onset type 1 diabetes mellitus, GADA-ELISA was positive in 56 participants and negative in nine participants (Table 2). For participants with slowly...

---

\(^{3}\) Oikawa \textit{et al.}

\(^{4}\) RSI Limited

\(^{5}\) Cosmic Corporation Co., Ltd, Tokyo, Japan

\(^{6}\) Cosmic Corporation Co., Ltd

\(^{7}\) Marketing authorization holder: Cosmic Corporation Co., Ltd, Tokyo, Japan

\(^{8}\) Marketing authorization holder: Cosmic Corporation Co., Ltd, Tokyo, Japan
Table 1 | Clinical characteristics of all participants with type 1 diabetes

| Clinical characteristics | Total (n = 95) | GADA-ELISA-positive (n = 74) | GADA-ELISA-negative (n = 21) | P-value |
|--------------------------|---------------|-------------------------------|-------------------------------|---------|
| Men, n (%)               | 31            | 22 (71%)                      | 9 (29%)                       | 0.258†  |
| AT1DM                    | 65/30         | 56/18                         | 9/12                          | 0.004   |
| Age (years)              | 43.7 ± 13.8   | 43.1 ± 13.6                   | 45.7 ± 14.7                   | 0.461   |
| BMI (kg/m²)              | 22.3 ± 3.6    | 21.9 ± 3.5                    | 24.0 ± 3.7                    | 0.022   |
| eGFR (mL/min/1.73 m²)    | 66.4 ± 40.5   | 65.4 ± 43.4                   | 70.0 ± 28.6                   | 0.643   |
| Duration of diabetes (years) | 13.3 ± 10.4 | 13.4 ± 10.6                   | 12.8 ± 10.0                   | 0.805   |
| Insulin dosage (units/kg/day) | 0.56 ± 0.28 | 0.56 ± 0.27                   | 0.53 ± 0.31                   | 0.658   |
| GADA-RIA (U/mL)          | 3.4 (2.1–5.9) | 3.85 (2.1–5.8)                | 2.9 (2.1–6.0)                 | 0.584‡  |
| GADA-ELISA (U/mL)        | 40.9 (9.0–91.1) | 51.5 (27.8–106.5)          | –                              |         |

Glutamic acid decarboxylase (GADA) measured by radioimmunoassay (GADA-RIA) and GADA measured by enzyme-linked immunosorbent assay (GADA-ELISA) are shown as the medians (interquartile ranges); other data are shown as the numbers of participants or means ± standard deviation. Student’s t-test. †The χ²-test. ‡Mann-Whitney U-test. AT1DM, acute-onset type 1 diabetes; BMI, body mass index; eGFR, estimated glomerular filtration rate; SPIDDM, slowly progressive insulin-dependent diabetes mellitus.

Comparison of HLA

The relationship between HLA and GADA-ELISA was analyzed for 52 participants whose HLA-A, HLA-B and HLA-DRB1 data were available, and 38 participants whose HLA-DQB1 data were available. There was no significant difference between positive and negative participants in either HLA class I genotype or haplotype. In all participants who had HLA class II allele DRB1*09:01, GADA-ELISA was positive (P = 0.0095, Table 3). In all participants who had DQB1*03:03, GADA-ELISA was also positive (P = 0.0356). Based on the haplotype, DRB1*09:01-DQB1*03:03 yielded a similar divide (P = 0.0357).

Among participants with acute-onset type 1 diabetes mellitus, the numbers of participants with DRB1*09:01, DQB1*03:03 or haplotype DRB1*09:01-DQB1*03:03 were not significantly

progressive insulin-dependent diabetes mellitus, GADA-ELISA was positive in 18 participants and negative in 12 participants. Among participants with slowly progressive insulin-dependent diabetes mellitus, p-CPI in the GADA-ELISA-negative group was significantly higher than that of the GADA-ELISA-positive group (r = 0.619, P = 0.002; Figure 2), although there was no significant difference between the two groups among participants with acute-onset type 1 diabetes mellitus.

GADA titer and disease duration

Disease duration was not significantly correlated with the GADA-RIA or GADA-ELISA titers in either acute-onset type 1 diabetes mellitus or slowly progressive insulin-dependent diabetes mellitus participants.
different between GADA-ELISA-positive and GADA-ELISA-negative participants ($P = 0.157$, $0.295$ and $0.539$, respectively). However, among participants with slowly progressive insulin-dependent diabetes mellitus, there was a significant difference in the number of participants with DRB1*09:01 between GADA-ELISA-positive and GADA-ELISA-negative participants (Table 4; $P = 0.021$).

**DISCUSSION**

In the present study, we investigated the relationships between GADA positivity measured by ELISA and clinical characteristics in Japanese people with type 1 diabetes. Among those with slowly progressive insulin-dependent diabetes mellitus and low-positive GADA titers by RIA, the GADA-ELISA-positive participants had significantly lower p-CPIs than the GADA-ELISA-negative participants, suggesting that endogenous insulin secretion was decreased, although there were no significant correlations between p-CPI and the duration of diabetes. Although the reason for this discrepancy is not clear, several hypotheses can be considered: the difference in GADA epitopes and the difference in affinity of GADA. There was a difference in the target antigen between two measurement methods: the target antigen of the RIA kit did not include the N-terminal region (aa 2–45), but that of the ELISA kit was full-length GAD. Therefore, the present findings might be related to a difference in the GADA epitope between the GADA-ELISA-negative and GADA-ELISA-positive participants. Kobayashi et al.\textsuperscript{10} reported an epitope unique to slowly progressive insulin-dependent diabetes mellitus in the N-terminal region (aa 1-83), and the time to becoming insulin dependent was shorter in people with antibodies to this epitope. Jin et al.\textsuperscript{11} reported that the fasting CPR value tended to be low in adults with latent autoimmune diabetes with antibodies to epitopes in the central and C-terminal regions. These studies suggest that there is a relationship between the GADA epitope and the endogenous insulin secretion capacity in people with slowly progressive insulin-dependent diabetes mellitus. It is unknown whether the discrepancy of epitopes between GADA-RIA and GADA-ELISA can be fully explained by the targeted amino acids. As proteins are folded into secondary structures and higher-order structures, reactivity to antibodies is not determined only by the amino acid sequence; that is, the primary structure.

Williams et al.\textsuperscript{12} reported that the GADA-ELISA kit showed better sensitivity and specificity than an N-terminally truncated
radiobinding assay GADA kit. They suggested that in the ELISA method, the antibody is sandwiched between GAD65 on the plate and GAD65 in solution, thereby preventing N-terminally restricted antibodies that are detected by many radiobinding assays from combining.

Another hypothesis is that the affinity of the GADA that can be detected is different. Kawasaki et al.\textsuperscript{13} measured GADA affinity with sera obtained from patients with GADA-RIA-positive/GADA-ELISA-negative and GADA-RIA-negative/GADA-ELISA-positive. They reported that GADA-RIA can detect both high- and low-affinity antibodies, but GADA-ELISA can detect only high affinity antibodies.

In the present study, a significant difference in endogenous insulin secretion was observed between GADA-ELISA-positive and GADA-ELISA-negative participants with slowly progressive insulin-dependent diabetes mellitus. Differences in GADA epitopes or affinity might reflect differences in cytotoxicity and lead to differences in endogenous insulin secretion capacity. In the present study, we did not carry out a detailed analysis on the difference in epitopes or the affinity between GADA-ELISA-positive and GADA-ELISA-negative participants. Further research is necessary on both of these issues.

In the present study, we found that participants with HLA-DRB1*09:01 or HLA-DQB1*03:03 were more likely to be associated with GADA-ELISA positivity. HLA-DRB1*09:01-DQB1*03:03 have been associated with type 1 diabetes in Japan and East Asia\textsuperscript{14}. These HLA haplotypes account for 14.3\% of the general population in Japan, but are rare in Caucasians\textsuperscript{14,15}. The nature of GADA in type 1 diabetes might be different between East Asians and Caucasians. In the present participants, there were no GADA-ELISA-negative participants with HLA-DRB1*09:01 or HLA-DQB1*03:03. It might be related to the divergence in HLA we reported and the divergence in the affinity of GADA Kawasaki et al.\textsuperscript{13} reported. A larger study is required to verify that GAD-antibody-positive people with these HLA genotypes will necessarily become positive on a GADA-ELISA.

At the onset of type 1 diabetes, it has been shown that cellular immunity, mainly T cells, is involved in the destruction of pancreatic \(\beta\)-cells\textsuperscript{16}. Itoh et al.\textsuperscript{17} reported that GADA-reactive T cells were significantly more numerous in people who are HLA-DR9-positive with type 1 diabetes. They suggested that HLA might be associated with differences in T-cell cytotoxicity to islet cells. We hypothesized that HLA genotypes are related to the rate of pancreatic \(\beta\)-cell destruction and GADA epitopes, which appear in the discrepancy between GADA-RIA and GADA-ELISA titers.

Yasui et al.\textsuperscript{18} reported that people with slowly progressive insulin-dependent diabetes mellitus who did not require insulin treatment for \(>5\) years after diagnosis had a significantly lower GADA titer in the RIA. It was suggested that slowly progressive insulin-dependent diabetes mellitus with low-titer-positive GADA-RIA is less likely to have lower insulin secretion, as insulin injection is not required and is a condition relatively close to type 2 diabetes. Among people with slowly progressive insulin-dependent diabetes mellitus with low-titer-positive GADA-RIA, the tendency of insulin secretion to decline in people who are GADA-ELISA-positive might indicate that GADA-ELISA is more predictive of faster progression to an insulin-dependent state. Considering that people who are GADA-ELISA-positive with slowly progressive insulin-dependent diabetes mellitus had more DRB1*09:01 and DQB1*03:03 HLA alleles, GADA-ELISA might reflect more strongly on the activity of T cells against pancreatic \(\beta\)-cells.

In the present study, no significant difference was observed in endogenous insulin secretion between the people who were GADA-ELISA-positive and GADA-ELISA-negative with acute-onset type 1 diabetes mellitus. Acute-onset type 1 diabetes mellitus is defined as diabetic ketosis or ketoacidosis within 3 months from the onset of hyperglycemic symptoms and requires continuous insulin therapy\textsuperscript{7}. People with type 1 diabetes in whom the endogenous insulin secretion is preserved at least to some extent are not applicable to these criteria and are classified as having slowly progressive insulin-dependent diabetes mellitus. Therefore, p-CPI might be inevitably low in people with acute-onset type 1 diabetes mellitus, explaining why there was no significant difference in p-CPI between the GADA-ELISA-positive group and GADA-ELISA-negative people.
There were several limitations to the present study. First, 1–3 years had passed from sample collection to measurement. For this kit, there was no information on the stability of the measured values >1 week after blood collection. However, as the specimens we used were preserved at −80°C, it was unlikely that there would be significant changes in the measured values over several years. Second, the timing of blood sampling and whether participants had fasted before sampling were not restricted. Many of our blood samples were taken in the post-prandial state, and one limitation was that the postprandial time was not the same. However, there have been some reports that CPI (p-CPI) after meals or glucose tolerance are more suitable as indicators of endogenous insulin secretion. Therefore, we used p-CPI as an evaluation of endogenous insulin secretion in the present study. Third, the number of participants whose HLA data were available was limited. Among the participants who could use the data, 52 were available for HLA-A, HLA-B and HLA-DRB1, and 38 were available for HLA-DQB1. Further studies using larger numbers with HLA data will be required. Finally, the present study was carried out in an ethnically homogeneous population, which might not be representative of the entire population with type 1 diabetes. It will be necessary to confirm whether these findings are universal beyond race using larger samples in the future.

In conclusion, in Japanese people with slowly progressive insulin-dependent diabetes mellitus participants with glutamic acid decarboxylase measured by enzyme-linked immunosorbent assay (GADA-ELISA) positivity and GADA-ELISA negativity are shown. SPIDDM participants with HLA-DRB1*09:01 were all GADA-ELISA-positive (7 vs 0, \( P = 0.021 \)). Fisher’s exact probability test. Susceptible to type 1 diabetes. HLA, human leukocyte antigen.

**Table 3** | Frequencies of human leukocyte antigen genotypes and haplotypes in all participants with glutamic acid decarboxylase measured by enzyme-linked immunosorbent assay positivity and negativity

| Genotype/haplotype | GADA-ELISA-positive | GADA-ELISA-negative | P-value |
|--------------------|---------------------|---------------------|---------|
| HLA-DRB1           |                     |                     |         |
| n                  | 41                  | 11                  |         |
| DRB1*0405†         | 15                  | 5                   | 0.7300  |
| DRB1*0802‡         | 9                   | 0                   | 0.1766  |
| DRB1*1501‡         | 18                  | 0                   | 0.0095  |
| DRB1*1502‡         | 1                   | 1                   | 0.3816  |
| HLA-DQB1           |                     |                     |         |
| n                  | 29                  | 9                   |         |
| DQB1*0401†         | 11                  | 4                   | 1       |
| DQB1*0302‡         | 10                  | 2                   | 0.6893  |
| DQB1*0303‡         | 12                  | 0                   | 0.0356  |
| DQB1*0602‡         | 1                   | 1                   | 0.4225  |
| DQB1*0601‡         | 6                   | 2                   | 1       |
| DRB1-DQB1 haplotype|                     |                     |         |
| n                  | 28                  | 9                   |         |
| 0405-0401†         | 10                  | 4                   | 0.7046  |
| 0802-0302‡         | 6                   | 0                   | 0.3205  |
| 0901-0303†         | 11                  | 0                   | 0.0357  |
| 1501-0602‡         | 1                   | 1                   | 0.4324  |
| 1502-0601‡         | 1                   | 1                   | 0.4324  |

Comparisons of the frequencies of HLA-DRB1 genotypes, HLA-DQB1 genotypes and DRB1-DQB1 haplotypes between type 1 diabetes participants with glutamic acid decarboxylase (GADA) measured by enzyme-linked immunosorbent assay (GADA-ELISA) positivity and GADA-ELISA negativity are shown. Fisher’s exact probability test. †Susceptible to type 1 diabetes. ‡Resistant to type 1 diabetes. HLA, human leukocyte antigen.

**Table 4** | Comparison of human leukocyte antigen genotypes and haplotypes between glutamic acid decarboxylase measured by enzyme-linked immunosorbent assay-positive and glutamic acid decarboxylase measured by enzyme-linked immunosorbent assay-negative participants with slowly progressive insulin-dependent diabetes mellitus

| Genotype/haplotype | GADA-ELISA-positive | GADA-ELISA-negative | P-value |
|--------------------|---------------------|---------------------|---------|
| HLA-DRB1           |                     |                     |         |
| n                  | 9                   | 5                   |         |
| DRB1*0405†         | 0                   | 2                   | 0.1099  |
| DRB1*0802‡         | 1                   | 0                   | 1       |
| DRB1*0901†         | 7                   | 0                   | 0.021   |
| DRB1*1501‡         | 0                   | 0                   | 1       |
| DRB1*1502‡         | 1                   | 1                   |         |
| HLA-DQB1           |                     |                     |         |
| n                  | 8                   | 5                   |         |
| DQB1*0401†         | 0                   | 2                   | 0.1282  |
| DQB1*0302‡         | 2                   | 1                   | 1       |
| DQB1*0303‡         | 5                   | 0                   | 0.0754  |
| DQB1*0602‡         | 0                   | 0                   | 1       |
| DQB1*0601‡         | 3                   | 2                   | 1       |
| DRB1-DQB1 haplotype|                     |                     |         |
| n                  | 8                   | 5                   |         |
| 0405-0401†         | 0                   | 2                   | 0.1282  |
| 0802-0302‡         | 1                   | 0                   | 1       |
| 0901-0303†         | 5                   | 0                   | 0.0754  |
| 1501-0602‡         | 0                   | 0                   | 1       |
| 1502-0601‡         | 1                   | 1                   |         |

Comparisons of the frequencies of HLA-DRB1 genotypes, HLA-DQB1 genotypes and DRB1-DQB1 haplotypes between slowly progressive insulin-dependent diabetes mellitus participants with glutamic acid decarboxylase measured by enzyme-linked immunosorbent assay (GADA-ELISA) positivity and GADA-ELISA negativity are shown. SPIDDM participants with HLA-DRB1*09:01 were all GADA-ELISA-positive (7 vs 0, \( P = 0.021 \)). Fisher’s exact probability test. †Susceptible to type 1 diabetes. ‡Resistant to type 1 diabetes. HLA, human leukocyte antigen.

**ACKNOWLEDGMENTS**

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. We thank Ms Konari for helping us prepare the serum samples.
DISCLOSURE
The authors declare no conflict of interest.

REFERENCES
1. American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2018. *Diabetes Care* 2018; 41: S13–S27.
2. Kawasaki E, Matsuura N, Eguchi K. Type 1 diabetes in Japan. *Diabetologia* 2006; 49: 828–836.
3. Oikawa Y, Tanaka H, Uchida J, et al. Slowly progressive insulin-dependent (type 1) diabetes positive for anti-GAD antibody ELISA test may be strongly associated with a future insulin-dependent state. *Endocr J* 2017; 64: 163–170.
4. Fujikawa K, Hata M, Kasai N, et al. Results of GAD antibody measurement by ELISA kit in cases with low antibody titer of GAD by RIA kit (Japanese). *J Jpn Diabetes Soc* 2017; 60: S255.
5. Imagawa A, Hanafusa T, Awata T, et al. Report of the committee of the Japan Diabetes Society on the research of fulminant and acute-onset type 1 diabetes mellitus: new diagnostic criteria of fulminant type 1 diabetes mellitus (2012). *Diabetol Int* 2012; 3: 179–183.
6. Jones AG, Hattersley AT. The clinical utility of C-peptide measurement in the care of patients with diabetes. *Diabet Med* 2013; 30: 803–817.
7. Kawasaki E, Maruyama T, Imagawa A, et al. Diagnostic criteria for acute-onset type 1 diabetes mellitus (2012). *Diabetol Int* 2013; 4: 221–225.
8. Tanaka S, Ohmori M, Awata T, et al. Diagnostic criteria for slowly progressive insulin-dependent (type 1) diabetes mellitus (SPIDDM) (2012): report by the committee on slowly progressive insulin-dependent (type 1) diabetes mellitus of the Japan Diabetes Society. *Diabetol Int* 2015; 6: 1–7.
9. Powell M, Prentice L, Asawa T, et al. Glutamic acid decarboxylase autoantibody assay using 125-labelled recombinant GAD65 produced in yeast. *Clin Chim Acta* 1996; 256: 175–188.
10. Kobayashi T, Tanaka S, Okubo M, et al. Unique epitopes of glutamic acid decarboxylase autoantibodies in slowly progressive type 1 diabetes. *J Clin Endocrinol Metab* 2003; 88: 4768–4775.
11. Jin P, Huang G, Lin J, et al. Epitope analysis of GAD65 autoantibodies in adult-onset type 1 diabetes and latent autoimmune diabetes in adults with thyroid autoimmunity. *Acta Diabetol* 2011; 48: 149–155.
12. Williams AJ, Lampasona V, Schlosser M, 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.
13. Kawasaki E, Okada A, Uchida A, et al. Discrepancy of glutamic acid decarboxylase 65 autoantibody results between RSR radioimmunoassay and enzyme-linked immunosorbent assay in patients with type 1 diabetes is related to autoantibody affinity. *J Diabetes Investig* 2019; 10: 990–996.
14. Ikegami H, Nosu S, Babaya N, et al. Genetic basis of type 1 diabetes: similarities and differences between east and west. *Rev Diabet Stud* 2008; 5: 64–72.
15. Ikeda N, Kojima H, Nishikawa M, et al. Determination of HLA-A, -B, -DRB1 allele and haplotype frequency in Japanese population based on family study. *Tissue Antigens* 2015; 85: 252–259. (In Japanese).
16. Nagata M, Yasuda H, Moriyama Y. Abnormality of cellular immunity in type 1 diabetes (Japanese). *J Jpn Diabetes Soc* 2009; 52: 679–682.
17. Itoh A, Shimada A, Kodama K, et al. GAD-reactive T cells were mainly detected in autoimmune-related type 1 diabetic patients with HLA DR9. *Ann N Y Acad Sci* 2004; 1037: 33–40.
18. Yasui J, Kawasaki E, Tanaka S, et al. Clinical and genetic characteristics of non-insulin-requiring glutamic acid decarboxylase (GAD) autoantibody-positive diabetes: a nationwide survey in Japan. *PloS ONE* 2016; 11: e0155643.
19. Meier JJ, Menge BA, Breuer TG, et al. Functional assessment of pancreatic β-cell area in humans. *Diabetes* 2009; 58: 1595–1603.
20. Okuno Y, Komada H, Sakaguchi K, et al. Postprandial serum C-peptide to plasma glucose concentration ratio correlates with oral glucose tolerance test- and glucose clamp-based disposition indexes. *Metabolism* 2013; 62: 1470–1476.