

Clinical heterogeneity of adult Japanese diabetes depending on titers of glutamic acid decarboxylase autoantibodies

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ABSTRACT

Aims/Introduction: We examined whether levels of glutamic acid decarboxylase autoantibodies (GADAb) might show the clinical heterogeneity of adult Japanese diabetes.

Materials and Methods: In this cross-sectional study, the serum levels of GADAb were measured in a total of 1857 consecutive adult diabetic patients aged 20 years or older. The patients with positive GADAb, arbitrarily defined as ≥ 1.5 U/mL, were divided into quartiles according to the number of patients. The age- and sex-matched diabetic patients without GADAb were selected as a control group.

Results: A total of 103 (5.5%) of the diabetic patients had GADAb, and showed higher HbA_{1c} and serum high-density lipoprotein (HDL) cholesterol levels, lower body mass index (BMI), urinary C-peptide immunoreactivity (CPR), serum triglycerides (TG) and uric acid (UA) levels, and lower prevalence of metabolic syndrome than the control group ($P < 0.05$). Quartiles 3 and 4 (i.e. GADAb ≥ 4.6 U/mL) showed a higher HbA_{1c} level, lower BMI, urinary CPR, serum TG and UA levels, quartile 2 ($2.5 \leq$ GADAb < 4.6 U/mL) showed a lower BMI level than the control group ($P < 0.05$). Among the clinical parameters, we observed significant upward trends for both HbA_{1c} and serum HDL cholesterol levels, and significant downward trends for BMI, serum TG and UA, urinary CPR levels, and prevalence of metabolic syndrome across GADAb quartiles ($P < 0.05$ for trend).

Conclusions: These results show that the clinical phenotype of adult Japanese diabetes correlates with GADAb levels, and that patients with GADAb (≥ 2.5 U/mL) show different characteristics from those without GADAb, although further longitudinal studies are required. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2011.00190.x, 2012)

KEY WORDS: Glutamic acid decarboxylase autoantibodies, Type 1 diabetes mellitus, Japanese

INTRODUCTION

Type 1 diabetes is characterized by the presence of markers of islet cell autoantibodies, such as autoantibodies to insulin, autoantibodies to glutamic acid decarboxylase (GADAb) and autoantibodies to the tyrosine phosphatases IA-2 and IA-2 β ¹. In Japan, positive GADAb titers have been defined as ≥ 1.5 U/mL, which corresponds to the mean + 3 SD value of a small number of Japanese normal controls². However, it is often difficult to

define type 1 and type 2 diabetes, as there is considerable heterogeneity of the clinical phenotype of adult diabetes with GADAb. Such cases with characteristics of both type 1 and type 2 diabetes are variably referred to as latent autoimmune diabetes in adults (LADA)³⁻⁵, slowly progressing to insulin-dependent diabetes (SPIDDM)⁶, non-insulin-requiring autoimmune diabetes⁷ or type 1.5 diabetes^{8,9}.

In the present study, we examined whether levels of GAD autoantibodies could show the clinical heterogeneity of adult Japanese diabetes.

MATERIALS AND METHODS

This was a cross-sectional study including a total of 1857 consecutive diabetic patients aged 20 years or older who attended our center between December 2000 and July 2007. The diagnosis of diabetes mellitus was made according to the criteria of the American Diabetes Association. The GADAb were examined at the first visit to our center. The serum GADAb titers were measured by a commercial radioimmunoprecipitation assay using

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¹²⁵I-labeled recombinant human GAD65 as a tracer reagent (Cosmic, Tokyo, Japan) and GADAb titers ≥ 1.5 U/mL (mean + 3 SD of Japanese normal controls) were judged as positive, according to the previous report². In the First Proficiency Evaluation of Diabetes Antibody Standardization Programs organized by the Immunology of Diabetes Society, this assay (Lab ID148) had 82% sensitivity and 92% specificity¹⁰. As GADAb showed a skewed distribution, the patients with positive GADAb were divided into four subgroups (quartiles) depending on the number of patients. Diabetic patients without GADAb who were matched for age and sex with those with GADAb were selected as a control group. The clinical characteristics, such as body mass index (BMI), systolic and diastolic blood pressure (BP), serum total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides (TG), uric acid (UA), HbA_{1c}, creatinine, C-reactive protein (CRP), urinary C-peptide immunoreactivity (CPR), urinary ketone and prevalence of metabolic syndrome, were compared among each group. The urinary C-peptide levels were initially measured by enzyme immunoassay (LS reagents "Eiken" C-peptide; Eiken Chemical, Tokyo, Japan) until June 2006, and later measured at our center by chemiluminescent immunoassay using ARCHITECT C-Peptide assay (Abbott Japan, Tokyo, Japan). The value of HbA_{1c}, which is equivalent to the internationally used HbA_{1c} (%) defined by the National Glycohemoglobin Standardization Program, is expressed by adding 0.4% to the HbA_{1c} (%) defined by the Japan Diabetes Society^{11,12}. In the present study, metabolic syndrome was defined as BMI ≥ 25 and two of the following: fasting plasma glucose ≥ 6.1 mmol/L or use of medication for hyperglycemia, dyslipidemia (TG ≥ 1.7 mmol/L or HDL cholesterol < 1.03 mmol/L) or use of a cholesterol-lowering agent and BP $\geq 130/85$ mmHg or use of antihypertensive agent. The present study has been approved by the ethics committee of Medical Research Institute Kitano Hospital.

Statistical Analysis

The results are expressed as frequencies or as means \pm SD. Frequency differences were compared using χ^2 -test or Fisher's exact test when appropriate. Statistical differences between groups for quantitative variables (BMI, systolic BP, diastolic BP, serum total cholesterol, HDL cholesterol, LDL cholesterol, TG, UA, HbA_{1c}, creatinine, CRP and urinary CPR) were investigated using multiple linear regressions. Comparisons were adjusted for sex, age on examination and treatment. Data of serum TG, HDL cholesterol and urinary CPR were transformed using log base 10 to normalize their distributions. Because GADAb showed a skewed distribution, the patients with positive GADAb were divided into quartile groups depending on the number of patients. Analyses of trends for variables across quartile groups of GADAb were carried out using Cuzick's non-parametric test for trend. A *P*-value of < 0.05 was considered to be statistically significant. All the analyses were carried out with SPSS 11.0 (SPSS, Chicago, IL, USA) and Intercooled Stata 10.0 (StataCorp, College Station, TX, USA).

RESULTS

Of the 1857 diabetic patients, a total of 103 (5.5%) diabetic patients were found to have GADAb. Table 1 shows clinical characteristics of patients with positive GADAb and those of control patients without GADAb. The mean age of diabetic patients with GADAb was 57 ± 16 years (male:female ratio 48:55). Compared with the control group without GADAb, the patients with GADAb showed a clinically different phenotype, including higher HbA_{1c} and serum HDL cholesterol levels, and higher frequency of urinary ketone, lower BMI, urinary CPR, serum TG and UA levels, and lower prevalence of metabolic syndrome (Table 1).

Figure 1 shows the distribution of GADAb titers in all diabetic patients with GADAb (≥ 1.5 U/mL; $n = 103$). As GADAb showed a skewed distribution, we divided all patients with GADAb ($n = 103$) into quartile groups, each of which had an equal number of patients ($n = 25$ or 26). The clinical characteristics of each quartile group are shown

Table 1 | Clinical characteristics of patients with positive glutamic acid decarboxylase autoantibodies and negative glutamic acid decarboxylase autoantibodies diabetes

	GADAb (-) diabetes	GADAb (+) diabetes
<i>n</i> (Female/male)	103 (55/48)	103 (55/48)
GADAb titer (U/mL)	< 1.5	$1.5 \leq$
Range	–	1.5–19900
Mean	–	777.5 ± 2854.1
Age (years)	57 ± 15	57 ± 16
BMI (kg/m ²)	25.1 ± 4.2	$22.7 \pm 4.3^{***}$
Systolic blood pressure (mmHg)	136 ± 22	132 ± 18
Diastolic BP (mmHg)	79 ± 11	78 ± 11
Total cholesterol (mmol/L)	5.38 ± 1.35	5.10 ± 1.05
HDL cholesterol (mmol/L)	1.38 ± 0.43	$1.53 \pm 0.43^*$
LDL cholesterol (mmol/L)	3.13 ± 1.11	2.89 ± 0.80
Triglycerides (mmol/L)	2.03 ± 1.40	$1.59 \pm 1.25^{***}$
Uric acid (μ mol/L)	329 ± 97	$287 \pm 136^*$
HbA _{1c} (%)	7.9 ± 2.5	$9.2 \pm 2.7^*$
Urinary CPR (nmol/24 h)	24.7 ± 17.6	$20.4 \pm 20.6^*$
Creatinine (μ mol/L)	77 ± 63	97 ± 166
CRP (mg/dL)	1.20 ± 3.81	0.88 ± 2.48
Urinary ketone (%)	8.5	19.2*
Metabolic syndrome (%)	39.2	21.4*
Treatment (%)		
Insulin \pm hypoglycemic agent	18.4	35.9**
Hypoglycemic agent	29.1	29.1
Antihypertensive agent	34.0	29.1
Cholesterol lowering agent	18.4	8.7*

Data are means \pm SD. All comparisons are adjusted for age of recruitment, sex and treatment.

BMI, body mass index; BP, blood pressure; CPR, C-peptide immunoreactivity; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

P* < 0.05 ; *P* < 0.01 ; ****P* < 0.005 vs negative glutamic acid decarboxylase autoantibodies (GADAb) diabetes.

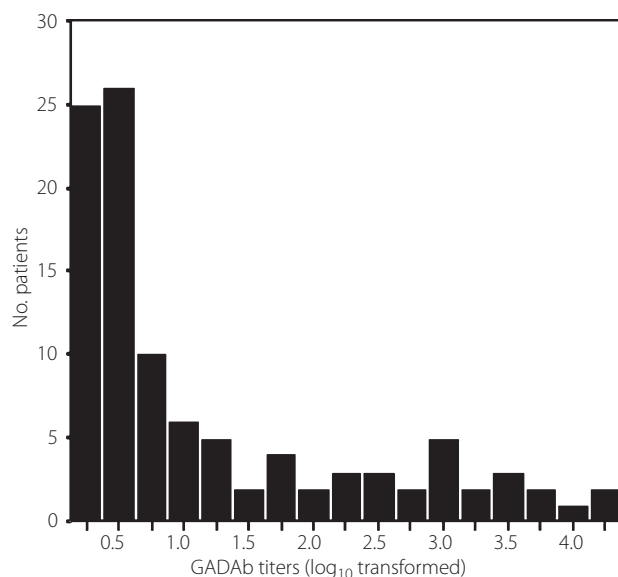


Figure 1 | Distribution of titers of glutamic acid decarboxylase autoantibodies (GADAb) in diabetic patients with GADAb.

in Table 2. Compared with the control group without GADAb, quartiles 3 ($4.6 \leq \text{GADAb} < 103 \text{ U/mL}$) and 4 ($103 \text{ U/mL} \leq \text{GADAb}$) showed a higher HbA_{1c} level, lower BMI, urinary CPR, serum TG and UA levels, and quartiles 2 ($2.5 \leq \text{GADAb} < 4.6 \text{ U/mL}$) showed a lower BMI level. In contrast, quartile 1 ($1.5 \leq \text{GADAb} < 2.5 \text{ U/mL}$) showed no significant differences from the control group (Table 2). Among the clinical parameters, we observed significant upward trends for HbA_{1c} and serum HDL cholesterol levels, and frequency of urinary ketone, whereas we observed significant downward trends for BMI, serum TG and UA, urinary CPR levels, and prevalence of metabolic syndrome across GADAb quartiles (Table 2).

DISCUSSION

The present study showed that diabetic patients with GADAb (defined as $\geq 1.5 \text{ U/mL}$) showed a significantly different phenotype from those without GADAb (defined as $< 1.5 \text{ U/mL}$). The patients with GADAb were characterized by reduced insulin secretion, and lower prevalence of metabolic syndrome and its components. Furthermore, quartiles 3

Table 2 | Clinical characteristics of patients with different titers of positive glutamic acid decarboxylase autoantibodies and negative glutamic acid decarboxylase autoantibodies diabetes

	GADAb (-) diabetes	GADAb (+) diabetes				P-value for trend across quartiles
		Quartile 1	Quartile 2	Quartile 3	Quartile 4	
n (Female/male)	103 (55/48)	26 (10/16)	26 (18/8)	26 (10/16)	25 (17/8)	NA
GADAb titer (U/mL)	<1.5	1.5 ≤ and <2.5	2.5 ≤ and <4.6	4.6 ≤ and <103.0	103.0 ≤	NA
Range	-	1.5-2.4	2.5-4.5	4.6-68.4	103.0-19900.0	NA
Mean	-	1.8 ± 0.3	3.2 ± 0.6	19.5 ± 19.9	3177.6 ± 5166.8	NA
Age (years)	57 ± 15	61 ± 11	59 ± 16	59 ± 16	50 ± 17*	0.222
BMI (kg/m ²)	25.1 ± 4.2	25.8 ± 4.8	22.2 ± 4.0*	20.9 ± 4.6**	22.1 ± 2.6***	<0.001
Systolic BP (mmHg)	136 ± 22	138 ± 17	139 ± 20	121 ± 8.3*	129 ± 21	0.071
Diastolic BP (mmHg)	79 ± 11	81 ± 10	78 ± 13	76 ± 8.6	77 ± 11	0.437
Total cholesterol (mmol/L)	5.38 ± 1.35	5.08 ± 1.16	5.21 ± 1.03	4.92 ± 1.06	5.15 ± 1.02	0.268
HDL cholesterol (mmol/L)	1.38 ± 0.43	1.33 ± 0.46	1.56 ± 0.47	1.64 ± 0.36*	1.61 ± 0.36	0.005
LDL cholesterol (mmol/L)	3.13 ± 1.11	3.07 ± 0.96	2.78 ± 0.75	2.74 ± 0.43	2.91 ± 0.90	0.152
Triglycerides (mmol/L)	2.03 ± 1.40	1.70 ± 0.98	1.69 ± 1.33	1.41 ± 1.30**	1.52 ± 1.41***	<0.001
Uric acid (μmol/L)	329 ± 97	350 ± 202	289 ± 100	267 ± 113*	234 ± 57***	<0.001
HbA _{1c} (%; NGSP value)	8.3 ± 2.5	8.6 ± 2.2	9.1 ± 2.6	9.6 ± 2.7*	10.6 ± 2.7***	<0.001
Urinary CPR (nmol/24 h)	24.7 ± 17.6	24.7 ± 18.8	23.1 ± 22.0	17.2 ± 26.5*	15.8 ± 12.7*	0.015
Creatinine (μmol/L)	77 ± 63	117 ± 123	102 ± 181	115 ± 258	53 ± 15	0.016
CRP (mg/dL)	1.20 ± 3.81	0.59 ± 1.10	1.53 ± 4.13	0.68 ± 1.48	0.66 ± 1.83	0.088
Urinary ketone (%)	8.5	4.2	12.0	20.0	40.0	<0.001
Metabolic syndrome (%)	39.2	46.2	18.8	7.7	14.3	0.008
Treatment (%)						
Insulin ± hypoglycemic agent	18.4	34.6	42.3*	34.6	32.0	0.030
Hypoglycemic agent	29.1	30.8	26.9	30.8*	28.0	0.685
Antihypertensive agent	34.0	46.2	34.6	26.9	8.0*	0.029
Cholesterol lowering agent	18.4	15.4	7.7	3.8	8.0	0.030

Data are means ± SD. All comparisons are adjusted for age of recruitment, sex and treatment.

BMI, body mass index; BP, blood pressure; CPR, C-peptide immunoreactivity; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NA, not applicable.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$ vs negative glutamic acid decarboxylase autoantibodies (GADAb) diabetes.

and 4 (i.e. GADAb ≥ 4.6 U/mL) also showed significantly different phenotypes, characterized by reduced insulin secretion and lower prevalence of components of metabolic syndrome, and quartile 2 ($2.5 \leq \text{GADAb} < 4.6$ U/mL) showed a lower BMI level, compared with the control group without GADAb. In contrast, quartile 1 ($1.5 \leq \text{GADAb} < 2.5$ U/mL) showed no significant differences. Among the clinical parameters, we observed significant upward trends for both HbA_{1c} and serum HDL cholesterol levels, and significant downward trends for BMI, serum TG and UA, urinary CPR levels, and prevalence of metabolic syndrome across GADAb quartiles. These results show that the clinical phenotype of adult Japanese diabetes correlates with the levels of GADAb: patients with high titers of GADAb showed the classical phenotype of type 1 diabetes characterized by reduced insulin secretion, and lower prevalence of metabolic syndrome and its components; in contrast, those with low GADAb titers showed the classical phenotype of type 2 diabetes with more prominent traits of metabolic syndrome. It is also shown in the present study that adult Japanese diabetic patients with GADAb levels higher than 2.5 U/mL show a different clinical phenotype from those without GADAb, although it remains to be elucidated whether patients show a different clinical course, such as insulin dependency according to GADAb levels.

Li *et al.* have recently reported that the optimal cut-off point to differentiate two subtypes of LADA characterized by insulin deficiency and metabolic syndrome, respectively, in Chinese patients was 175 U/mL, based on the receiver-operator characteristic (ROC) curve analysis^{13,14}. Buzzetti *et al.* from Italy reported that a GADAb titer of 32 arbitrary units could be the cut-off value, based on a bimodal distribution of titers of GADAb¹⁵. In Japan, a prospective study with 54 Japanese adult diabetic patients suggested that GADAb levels higher than 10 U/mL (180 WHO U/mL) could predict a risk for progression to insulin requirement for a mean of 4 years, based on the ROC curve analysis^{16,17}. There are several different units to measure GADAb, which makes it difficult to compare different studies. The use of standardized units as WHO U/mL is expected to overcome those problems^{10,18,19}.

The present study is limited by several factors. First, it is a cross-sectional study. Second, it is a single center study with a moderate number of patients. Third, it included only a small number of patients with positive GADAb (≥ 1.5 U/mL), reflecting a low frequency of type 1 diabetes in Japanese patients. Fourth, the prevalence of other islet autoantibodies (IA-2Ab, IAA, ZnT8Ab) was not examined. Finally, the GADAb were examined at the first visit, excluding potential confounding factors, such as the effects of duration of diabetes. Further longitudinal studies using standardized units as WHO U/mL are justified in order to elucidate whether diabetic patients show a different course of insulin deficiency in the long term depending on GADAb titers.

In summary, the present study shows that titers of GADAb can reveal the clinical heterogeneity of adult Japanese diabetes: the clinical phenotype of diabetes correlates with GADAb levels, and patients with higher GADAb levels show different characteristics from those without GADAb.

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The authors have no relevant conflict of interest to disclose.

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