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The clinical and immunogenetic characteristics of adult-onset type 1 diabetes mellitus in Korea

Abstract Although the HLA class II alleles and immunological abnormalities are associated with type 1 diabetes mellitus (T1DM) in all racial groups, there are considerable variations in the genotypes and the prevalence of autoantibodies. In order to investigate the characteristics of the immunogenetic patterns and to use these as an early diagnostic tool and guideline for a therapeutic plan, we examined the clinical characteristics and the patterns of anti-GAD antibody (GADA), IA-2 antibody (IA-2A), HLA-DR and HLA-DQ in Korean adult-onset T1DM patients. Adult-onset patients had higher serum C-peptide levels than child-onset patients. In adult-onset patients, the prevalence of GADA and IA-2A were 59.5% and 15.3% respectively, and increased frequencies of HLA-DR4 and -DR9 were found. The frequencies of HLA-DQA1, -DQB1 and -DQ heterodimers were similar to those of the control, but child-onset patients had high frequencies of the HLA-DR3, -DR4, -DR9, DQA1*0301, DQA1*0501 and DQB1*0201 genotypes. In conclusion, Korean adult-onset T1DM patients had a lower prevalence of GADA, which was comparable to that found in Caucasian patients. The detection of GADA might help to predict the insulin dependency of adult-onset diabetes. Difference in the frequencies of diabetes associated with HLA type suggests that there might be a heterogeneity in the pathogenesis of diabetes according to the age of onset.

Key words Adult-onset type 1 DM · LADA · Anti-GAD antibody · HLA-DR · HLA-DQ

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Introduction

Genetic and environmental factors are known to be involved in the autoimmune pathogenesis of type 1 diabetes mellitus (T1DM) [1–3], and the genetic factors have been report to contribute as much as 30%–60% to the pathogenesis of T1DM [4]. One of the most important genetic factors is the polymorphism of human leukocyte antigen (HLA) on the short arm of chromosome 6 [1, 3, 5].

Among various autoantibodies related to T1DM, islet cell cytoplasmic antibodies (ICA) are autoantibodies against the entire cytoplasm of islet cells. They can be used to determine the type of diabetes when measured with C-peptide levels [6]. However, there are several shortcomings to this method, as it is difficult and expensive to measure ICA levels, and its false positivity rate is reported to be as high as 20%–30% [7].

Both glutamic acid decarboxylase 65 and insulinoma-associated protein-2 are two important islet-specific autoantigen components of ICA. The simultaneous measurement of these antibodies was used as an alternative to ICA, and the sensitivity and specificity of diagnosis was increased accordingly [8–10]. However, racial and ethnical differences were apparent in the positivity of these autoantibodies [11]. In Korean and other Asian diabetes patients, the positive rate of GADA is reported to be lower than that of Caucasians [12–16].

Latent autoimmune diabetes of adult (LADA) is a subtype of T1DM, which accounts for 20% of T1DM cases [17–19]. Because clinical manifestations progress slowly and typical features of T1DM do not occur in every case, the initial treatment often involves diet and exercise or an oral hypoglycaemic agent, as is administered in cases of type 2 diabetes mellitus (T2DM). Immunogenetic markers can be helpful in the classification and determination of the treatment modality in these patients.

Thus, the aims of this study were to investigate the clinical characteristics, the frequency of HLA type and the prevalence of autoantibodies in Korean adult-onset T1DM and to compare these results with those of child-onset patients.

Research design and methods

Subjects

Two hundred and thirty-three patients who were diagnosed as T1DM at Yonsei University College of Medicine between 1991 and 1998 were recruited for this study. According to National Diabetes Data Group criteria [19], the patients were diagnosed as T1DM with (1) fasting C-peptide levels of less than 0.6 ng/ml on two occasions at stable state, (2) a past history of diabetic ketoacidosis (DKA) or frequent ketonuria, (3) a body mass index (BMI) of less than 24 kg/m^2 and (4) an insulin requirement for the control of DKA or hyperglycaemia. As controls, healthy adults who were more than 20 years old and without a past history or a family history of diabetes were selected. HLA-DR antigen typing was performed on 276 people, HLA-DQA1 antigen typing on 106 people and HLA-DQB1 antigen typing on 104 people. The patients were diagnosed as LADA when (1) they had anti-GAD antibody (GADA, >5 U/ml), (2) their ages at onset of diabetes were over 35 years and (3) they did not initially (at least 6 months) require insulin. The study protocol was approved by the Yonsei University College of Medicine ethical committee, and informed consent was obtained from each participant.

Methods

Clinical characteristics and biochemical profiles
The onset age of diabetes, duration of insulin treatment, past history of DKA and family history of diabetes were examined. Fasting and postprandial 2-h plasma glucose and C-peptide levels were measured. Plasma glucose was measured using the hexokinase methods, haemoglobin A1c (HbA1c) level was measured by electrophoresis and plasma c-peptide level was measured by radioimmunoassay (Incstar Co., Stillwater, MN, USA).

Measurement of GADA and IA-2A
GADA were determined by anti-GAD RIA kit (RSR, Cardiff, UK). Experimental procedures for GADA analysis have been described previously [20]. Briefly, 20 µl of test serum samples was first incubated with 50 µl ^{125}I-labelled human recombinant GAD65 for 2 h at room temperature. This was followed by the addition of 50 µl solid phase protein A, incubating for 1 h at room temperature to precipitate the labelled GAD–GADA complexes. After 30 min of 1500 g centrifugation at 4°C, the supernatants were discarded and the precipitates were counted for ^{125}I. Sera were considered GADA-positive if they contained >1 U/ml of antibody, which was more than 4 SD above the mean of healthy individuals recommended by RSR limited.

The intracellular domain of IA-2 (IA-2ic, amino acids 604–979), the amino-terminus of IA-2ic (IA-2icN, amino acids 604–776) and the carboxyl-terminus of IA-2ic (IA-2icC, amino acids 771–979) were synthesised *in vitro* with a TNT-coupled rabbit reticulocyte transcription/translation system (Promega, Madison, WI) in the presence of [^{35}S]methionine (>3000 mCi/mmol, Amersham, Arlington Heights, IL). The reticulocyte lysate was purified by spin column to remove free amino acids. The procedure of Grubin et al. [21] was followed with slight modification. Briefly, ~20 000 cpm of radiolabelled polypeptides was incubated with 3 µl of a serum sample overnight at 4°C on a rotating platform in 100 µl precipitation buffer (20 mmol/l Tris–HCl, pH 7.5, 150 mmol/l NaCl and 1% Triton X-100). Fifty microlitres of protein A–agarose (Life Technologies, Gaithersburg, MD) was added and the incubation was continued for another hour. After washing four times with the precipitation buffer, the beads were transferred to scintillation vials for counting. The data were presented as an average of two different experiments. Mean+3 standard deviation (SD) of the radioactivity detected in 51 normal control subjects was used as the cut-off level. The assay for the detection of IA-2A has been evaluated in the IA-2 Antibody Proficiency Test, which consistently scored over 90% of diagnostic sensitivity and specificity for type 1 diabetes.

HLA-DR and -DQ determination
HLA-DR and -DQ typing were performed using the lymphocyte microcytotoxicity method (One Lamda Co., Stamford, CT, USA).

HLA-DQA1 and -DQB1 determination
DNA was isolated from peripheral blood and amplified by the polymerase chain reaction. HLA-DQA1 was analysed by restriction fragment length polymorphism. HLA-DQB1 genotyping was done by dot blot hybridisation with sequence-specific oligonucleotides.

Statistics
Data were expressed as means±standard deviation. The mean value and the frequency of the diabetic and the control groups were compared using the *t*-test, Chi-square test and Fisher's exact test. The correlation between two autoantibodies was expressed as the π coefficient of correlation. Statistical analyses were conducted using SPSS for Windows version 11.0 (SPSS Inc., Chicago, IL, USA), and the level of significance used was $p<0.05$.

Results

Clinical characteristics of the subjects

Characteristics of patients
Average onset age was in fact 25.8 years of age. The gender ratio was 101:132 (M:F) and the average BMI was 19.9±2.8 kg/m^2. The positive rate of GADA was 59.7%, and IA-2A rate was 17.6%.

Characteristics according to onset age
There were no meaningful differences between the child-onset patient and adult-onset group with respect to sex, family history of diabetes, duration of diabetes, fasting plasma glucose, postprandial 2-h glucose or HbA1c (Table 1). But the adult-onset group had a lower incidence of ketonuria (67/123 *vs.* 65/94, *p*=0.04) and a higher BMI (20.4±2.9 *vs.* 18.0±2.4 kg/m^2, *p*=0.036) compared to those of the child-onset group. Also, fasting and postprandial 2-h C-peptide levels were higher in the adult-onset patients (0.66±0.43 *vs.* 0.53±0.35 µg/l, *p*=0.047; 0.88±0.77 *vs.* 0.68±0.45 µg/l, *p*=0.0001, respectively) (Table 1).

LADA patients
LADA patients accounted for 15.0% (35/233) of the T1DM patients. Compared to acute-onset patients in adult-onset T1DM patients, LADA patients showed no significant differences in age, sex, family history of DM, BMI, or the duration of DM, plasma glucose, HbA1c and history of ketonuria. However, they experienced onset later (33.5±11.3 *vs.* 41.3±13.4 years, *p*=0.004) and had higher fasting and postprandial 2-h C-peptide levels (0.55±0.32 *vs.* 0.83±0.58 µg/l, *p*=0.002; 0.43±0.04 *vs.* 1.25±1.21 µg/l, *p*=0.001, Table 2).

Immunologic characteristics based on the GADA and IA-2A positivity

GADA was positive in 59.7% (114 of 191) of all T1DM patients, 59.5% (66 of 111) of adult-onset patients and 60.0% (48/80) of child-onset patients. The GADA positiv-

ity was not different according to onset age or sex. On the other hand, the total T1DM patient group showed an IA-2A positivity of 17.6%, while child-onset patients showed 19.8% and adult-onset patients 15.3%. There was no significant difference in the IA-2A positivity according to onset age or sex. The positive rate of the GADA and IA-2A had a tendency to decrease with a shorter duration of DM, but this was not significant. Among the total T1DM patients, the concordance rate of these two autoantibodies was 52.2% (58/111) and π coefficient of correlation was 0.26 (*p*=0.004).

When we compared the clinical characteristics of 66 GADA-positive patients with those of 45 GADA-negative patients in the adult-onset patient group, no differences were found in family history of DM, frequency of ketonuria and BMI. However, the GADA-positive group had shorter duration of DM (9.7±4.3 *vs.* 7.9±3.5 years, *p*=0.034) and significantly higher fasting and postprandial 2-h C-peptide levels (0.74±0.50 *vs.* 0.48±0.22 ng/ml, *p*=0.002; 1.02±0.96 *vs.* 0.65±0.40 ng/ml, *p*=0.022).

Distribution of HLA-DR and -DQ antigen type and the HLA-DQ genotype

HLA-DR and -DQ antigen type
On comparing HLA-DR antigen type with the control group, DR3, DR4 and DR9 were found to be present at high frequencies in the child-onset group. However, in the adult-onset patient group, DR3 was not present at such a high frequency while DR4 and DR9 were higher than in the control group (107/276 *vs.* 55/102, *p*=0.01;

Table 1 Clinical characteristics of childhood and adult-onset T1DM patients

	Child-onset (*n*=105)	Adult-onset (*n*=128)	*p* value
Age (years)	20.1±8.7	45.0±13.9	–
Sex (male:female)	43:62	58:70	NS
Age of onset (years)	12.4±6.3	36.3±11.8	–
Family history of diabetes (%)	24/95 (25.0)	46/128 (35.9)	NS
BMI (kg/m^2)	18.0±2.4	20.4±2.9	0.036
Duration of diabetes (years)	7.7±5.5	8.8±6.8	NS
Plasma glucose (mmol/l)			
Fasting	9.29±2.40	10.06±2.11	NS
Postprandial 2 h	12.44±3.09	12.99±3.70	NS
Plasma C-peptide (µg/l)			
Fasting	0.53±0.35	0.66±0.43	0.047
Postprandial 2 h	0.68±0.45	0.88±0.77	0.0001
HbA1c (%)	9.29±2.07	9.53±1.8	NS
Ketonuria (%)	65/94 (69.1)	67/123 (54.5)	0.04
LADA (%)	0/99 (0.0)	35/128 (27.3)	0.00001

Values are mean±SD. *NS*, not significant

Table 2 Clinical characteristics of LADA and acute-onset T1DM patients in adult-onset T1DM

	LADA (*n*=35)	Acute-onset (*n*=93)	*p* value
Age (years)	46.4±13.5	41.1±13.8	NS
Sex (male:female)	17:18	36:34	NS
Age of onset (years)	41.3±13.4	33.5±11.3	0.004
Family history of diabetes (%)	11/35 (31.4)	36/93 (38.7)	NS
BMI (kg/m^2)	20.4±3.3	20.3±2.9	NS
Duration of diabetes (years)	5.1±2.9	7.7±6.1	NS
Plasma glucose (mmol/l)			
Fasting	9.66±2.38	9.39±1.97	NS
Postprandial 2 h	11.71±3.58	13.53±3.58	NS
Plasma C-peptide (µg/l)			
Fasting	0.83±0.58	0.55±0.32	0.002
Postprandial 2 h	1.25±1.21	0.43±0.04	0.0001
HbA1c (%)	9.0±1.8	9.7±1.9	NS
Ketonuria (%)	16/35 (45.7)	30/93 (32.3)	NS

Values are mean±SD. *NS*, not significant

Table 3 Frequencies of HLA-DR serotypes in childhood-onset and adult-onset T1DM patients

HLA-DR	Control (*n*=276)	Child-onset T1DM (*n*=87)			Adult-onset T1DM (*n*=102)		
	No. (%)	No. (%)	*p*	OR	No. (%)	*p*	OR
1	25 (9.1)	5 (5.7)	NS	–	13 (12.7)	NS	–
2	53 (19.2)	6 (6.9)	0.007	0.82	12 (11.8)	NS	–
3	17 (6.1)	22 (25.3)	0.0001	1.83	12 (11.8)	NS	–
4	107 (38.8)	45 (51.7)	0.035	1.14	55 (53.9)	0.01	1.19
5	52 (18.8)	10 (11.5)	NS	–	17 (16.7)	NS	–
6	56 (20.3)	9 (10.3)	0.037	0.88	22 (21.6)	NS	–
7	56 (20.3)	10 (11.5)	NS	–	15 (14.7)	NS	–
8	34 (12.3)	12 (13.8)	NS	–	9 (8.8)	NS	–
9	44 (15.9)	26 (29.9)	0.008	1.26	26 (25.5)	0.038	–
10	7 (2.5)	4 (4.6)	NS	–	3 (2.9)	NS	–
3 or 4	120 (43.5)	58 (66.7)	0.0001	1.25	63 (61.8)	0.002	1.22
3/4	4 (1.4)	10 (11.5)	0.0001	2.73	10 (9.8)	0.001	2.61
3 or 9	60 (21.7)	44 (50.6)	0.001	2.95	36 (35.3)	0.0001	2.39
3/9	1 (0.4)	5 (5.7)	0.004	4.62	2 (2.0)	NS	–
4 or 9	138 (50.0)	65 (74.7)	0.0001	1.27	72 (70.6)	0.0001	1.25
4/9	13 (4.7)	6 (6.9)	NS	–	9 (8.8)	NS	–

Data are number (%) of patients and control subject. *p* values *vs.* control group. *NS*, not significant

44/276 *vs.* 26/102, *p*=0.038, Table 3). The frequencies having a DR3/4 heterodimer or a haplotype of DR3 or DR4, DR3 or DR9, and DR4 or DR9 were higher in the adult-onset group or child-onset group than in the control group (Table 3). However, the frequencies of DQ antigen types were not different from the control group (data are not shown). In HLA-DR antigen type with positive GADA, in adult-onset patients, DR4 and DR9 were present at higher frequencies than in the normal control group.

HLA-DQ genotype
DQA1*0301 and DQA1*0501, which were present at high frequencies in the child-onset group, were not as frequent in the adult-onset group, while DQA1*0601 was present at a lower frequency in the child-onset and the adult-onset groups (Table 4). In contrast, none of the DQB1 genotype showed high frequency in the adult-onset group, and the frequencies of DQB1*0301 and DQB1*0601 genotypes were as low as in the child-onset group. In the child-onset group, the DQB1*0201 genotype appeared at high frequency (Table 5).

Table 4 Frequencies of HLA-DQA1 genotypes in child-onset and adult-onset T1DM patients

HLA-DQA1	Control (n=106)	Child-onset T1DM (n=52)			Adult-onset T1DM (n=95)		
	No. (%)	No. (%)	p	OR	No. (%)	p	OR
0101, 2	43 (40.6)	14 (26.9)	NS		32 (33.7)	NS	
0103	13 (12.3)	2 (3.8)	NS		8 (8.4)	NS	
0201	17 (12.3)	4 (7.7)	NS		14 (14.7)	NS	
0301	64 (60.4)	42 (80.8)	0.012	1.34	65 (68.4)	NS	
0501	29 (27.4)	24 (46.2)	0.021	1.34	21 (22.1)	NS	
0601	12 (11.3)	0 (0.0)	0.009	0.64	2 (2.1)	0.012	0.59

Data are number (%) of patients and control subject. *p* values *vs.* control group. *NS*, not significant

Table 5 Frequencies of HLA-DQB1 genotypes in childhood-onset and adult-onset T1DM patients

HLA-DQB1	Control (n=104) No. (%)	Child-onset T1DM (n=50)			Adult-onset T1DM (n=94)		
		No. (%)	p	OR	No. (%)	p	OR
0201	20 (19.2)	19 (38.0)	0.017	1.42	20 (21.3)	NS	
0301	31 (29.8)	4 (8.0)	0.002	0.69	13 (13.8)	0.017	0.69
0302	28 (26.9)	20 (40.0)	NS		26 (27.7)	NS	
0303	20 (19.2)	15 (30.0)	NS		24 (25.5)	NS	
0401	18 (17.3)	14 (28.0)	NS		21 (22.3)	NS	
0402	8 (7.7)	0 (0.0)	0.05	0.66	5 (3.3)	NS	
0501	13 (12.5)	3 (6.0)	NS		12 (12.8)	NS	
0502	7 (6.7)	0 (0.0)	NS		5 (3.3)	NS	
0503	7 (6.7)	4 (8.0)	NS		3 (3.2)	NS	
0601	23 (22.1)	0 (0.0)	0.001	0.63	4 (4.3)	0.0001	0.59
0602	7 (6.7)	1 (2.0)	NS		9 (9.6)	NS	
0603	1 (9.6)	1 (2.0)	NS		4 (4.3)	NS	
0604	17 (16.3)	10 (20.0)	NS		9 (9.6)	NS	

Data are number (%) of patients and control subject. *p* values *vs.* control group. *NS*, not significant

None of the HLA-DQ genotypes appeared at high frequency in the GADA-positive, adult-onset group, and the frequencies of DQA1*0601, DQB1*0301 and DQB1*0601 were lower in the normal control group.

Each of the DQA1 and DQB1 alleles were classified according to their status with respect to Arg52/non-Arg 52 and Asp 57/non-Asp 57. The expression of Arg52 homodimer in DQA1 was higher in the child-onset group than in the control group. However, no such difference was evident in the adult-onset group, and the expression of non-Asp 57 homodimer in DQB1 was increased in both the child-onset and the adult-onset groups (Table 6).

Susceptible heterodimer, which can be formed from both HLA-DQA1 and -DQB1, was analysed. It was discovered that those with both DQA1*Arg-52 and DQB1*nonAsp-57 alleles had an odds ratio (OR) of 1.4 in the child-onset group and of 1.3 in the adult-onset group, compared to those of the control group, which is not sig-

nificantly different. Those with the DQA1*nonArg-52 and DQB1*nonAsp-57 homodimers had an OR of 2.4 compared to the controls.

Among DQA1-DQB1 heterodimers, the frequency of DQA1*0301-DQB1*0201was found to be higher in the child-onset group, while no difference was observed in the adult-onset group (Table 7), and no difference was found in the frequency of heterodimer in the GADA-positive, adult-onset group.

Relationship between GADA and the HLA genotype

In the child-onset group, patients with risk factors for HLA presented higher positive rates for GADA than patients with no risk factors. In particular, a significant difference was noticed in patients carrying DQA1*0501-

Table 6 A comparison of the distributions of the HLA-DQA1 and DQB1 genotypes in child-onset and adult-onset T1DM patients

Genotype	Control	Child-onset T1DM			Adult-onset T1DM		
	No. (%)	No. (%)	p	OR	No. (%)	p	OR
DQA1	n=106	n=52	n=95				
Arg-52/Arg-52	44 (41.5)	32 (61.5)	0.027	1.31	42 (44.2)	NS	
Arg-52/nonArg-52	46 (43.4)	17 (32.7)	NS		37 (38.9)	NS	
nonArg-52/nonARg-52	16 (15.1)	3 (5.8)	NS		16 (16.8)	NS	
DQB1	n=104						
nonAsp-57/nonAsp-57	15 (14.4)	17 (34.0)	0.006	1.57	29 (30.9)	0.006	1.71
nonASP-57/Asp-57	55 (52.9)	4 (18.0)	NS		42 (44.7)	NS	
Asp-57/Asp-57	34 (32.9)	9 (48.0)	NS		23 (24.5)	NS	

Table 7 Frequencies of HLA-DQA1-DQB1 heterodimers in child-onset and adult-onset T1DM patients

DQA1-DQB1	Control (n=103) No. (%)	Child-onset T1DM (n=47)			Adult-onset T1DM (n=91)		
		No. (%)	p	OR	No. (%)	p	OR
0301-0201	8 (7.8)	13 (27.7)	0.002	1.93	11 (12.1)	NS	
0301-0302	24 (23.3)	19 (40.4)	NS		22 (24.2)	NS	
0301-0303	17 (16.5)	15 (31.9)	0.029	1.42	23 (25.3)	NS	
0301-0401	17 (16.5)	13 (27.7)	NS		21 (23.1)	NS	
0501-0201	8 (7.8)	12 (25.5)	0.008	1.83	11 (12.1)	NS	
0501-0302	4 (3.9)	11 (23.4)	0.004	2.07	5 (5.5)	NS	
0501-0303	2 (1.9)	4 (8.5)	0.009	3.39	3 (3.3)	NS	
0102-0601	10 (9.7)	0 (0.0)	NS		1 (1.1)	NS	

DQB1*0201 (9/11 *vs.* 10/25, p=0.031). However, when we compared the positive rates of GADA in the adult-onset group after classifying them by HLA risk factor, no difference was observed.

When we compared DR4-DQA1*0301-DQB1*0401 (DR4-DQ4) and DR9-DQA1*0301-DQB1*0303 (DR9-DQ9), which are common in adult-onset diabetic patients, and are homodimers formed due to linkage disequilibrium with DR4 and DR9, no difference was found in terms of the clinical characteristics. The positive rate of GADA was higher in the DR9-DQ9 group than in the DR4-DQ4 group (13/24 *vs.* 15/16, p=0.002).

Discussion

It has been reported that adult-onset patients (onset age of over 20) account for 50% of T1DM patients [22], and our study showed a similar ratio of 54.9% (128 out of 233 patients). Compared to child-onset patients, these patients had a lower incidence of ketonuria history, and many of them showed less typical forms of T1DM. In addition, they had higher plasma C-peptide levels, suggesting a better-preserved beta-cell function. These findings are consistent with a previous report [23], which stated that the

adult-onset patient group had a longer pre-diagnostic symptomatic period and a higher plasma C-peptide level.

In our study, patients with LADA represented 15.0% of all T1DM patients and this was lower than the 20% quoted in a Japanese report [17]. Korean patients with LADA had higher C-peptide levels than typical adult-onset T1DM patients, implying better preserved pancreatic beta-cell function. Because the onset age was later than typical T1DM, manifestations of T1DM were likely to appear at a later period. The gradual loss of pancreatic beta-cell function in Korean adult-onset T1DM would lead to an ambiguity in the determination of the type of DM at an early stage. As a consequence, many cases would be misdiagnosed as T2DM and treated without insulin.

As it was difficult to differentiate the types of adult-onset DM patients based on their clinical characteristics, the autoantibody was used as a marker. Moreover GADA was rarely found in normal people, and its positive rate does not change according to the age of onset or the duration of disease [24]. Due to these advantages, GADA is widely used as an index for T1DM. In the past, it has been generally accepted that the positive rate of GADA is less than 50% in the eastern hemisphere, for example, Korea was reported to show 5–30% [16, 25]. Such differences between East and West were thought to be due to the exis-

tence of non-autoimmune mechanisms [15]. With improved methods of measuring GADA, the positive rate of GADA was reported to be 50%–60% in Korea [25]. In our study, GADA was positive in 59.7% of T1DM patients.

IA-2A was also present in a high percentage (65%–75%) of newly developed T1DM patients in the West [26, 27], but in Japan and Asia [28], the positive rates were lower. In this study, 17.6% of all T1DM patients, 15.3% of adult-onset patients and 19.8% of child-onset patients were positive. The lower positive rate observed in our study may be explained by the characteristics of IA-2A, which decreases with late onset and longer duration [14, 29, 30]. Our study population might have involved more adult-onset patients and patients with longer disease durations than other studies. In our study, in 6 patients who had less than 1 year of duration, 33.3% positivity was observed, which was higher than the positive rate of total T1DM patients (dada are not shown).

It seems reasonable to conclude that GADA would be a more useful marker for T1DM than IA-2A, as LADA accounts for as much as 15%–20% of T1DM. Combining the report that suggested that GADA is a marker for adult-onset patients with ambiguous DM typing [23], and another report, which stated that GADA's positivity is independent of the onset age [13], it is perhaps reasonable to suggest that GADA should be used as a predictive marker for Korean diabetes patients who are initially diagnosed as T2DM after 20 years of age but later slowly progress to T1DM.

In our study, DR3, DR4 and DR9 were found to be present at high frequencies in the child-onset group and DR4 and DR9 were higher than control group in the adult-onset patient group. Previous data on HLA class II genes as a possible aetiological factor for T1DM was based on Caucasians. Our results differed from those of Caucasians, who expressed increased DR3 and DR4 [31, 32], but coincided with those of several investigations in Japanese [32–34]. Our results also differed from those in a Chinese study, which found that patients with an onset age of 20 years or less had high frequencies of DR3 and DR9 [35]. Interestingly, DR9 had a high frequency only in Oriental T1DM patients.

When we compared HLA-DQA1, the pattern was not found to be different in the GADA-positive adult-onset patient group and the total adult-onset T1DM patients. DQB1*0301, an increased factor in Japan, was at a higher frequency than the control in both the adult-onset and child-onset patient groups, but the difference was not statistically significant.

In addition, factors carrying Arg 52 on the DQ alpha-chain – DQA1*0301, 0401, 0501 and 0601, and factors carrying Asp 57 on the DQ beta-chain – DQB1*0201, 0302, 0304, 0501, 0502, 0504 and 0604, are known to be related to susceptibility in Caucasians [36–38]. However,

this did not hold true in Japan [39, 40], and our study result differed from that of the Japanese report above. For example, in the child-onset patient group, the frequencies of homodimer with Arg52 positive on the alpha chain and homodimer with Asp57 negative on the beta chain were high, which is consistent with a previous report, which showed highest risk associates with the combination of alpha-chain of the Arg-positive homodimer and the beta-chain of the Asp-negative homodimer, among the various combinations of DQ alpha chains and beta chains [41]. In contrast, in the adult-onset patient group, no difference was found in the Arg52-positive alpha chain homodimer, but the Asp 57-negative beta chain homodimer was present at a higher frequency, indicating the importance of the Asp57 beta chain on the determination of susceptibility to DM. However, unlike a report which stated that even when both alpha and beta chains had sensitivity factor, the susceptibility to DM depends on the number of heterodimers that bound cis or trans [41, 42], in the present report no differences were seen based on the number of heterodimers [36].

As the increased pattern of DQA1*0301, 0501 and DQB1*0201 in the child-onset patient group resembled study results in Caucasians, we presume these genetic factors are involved in the pathogenesis of DM, and that the possibility that these factors form a heterodimer and act as a DM aetiological factor are accordingly increased. Possibly, the adult-onset patient group is affected by other genetic factors in disequilibrium with DQA1 and DQB1 to induce autoimmunity.

As the DQ molecule is an antigen with diversified alpha and beta chains, and the Asp 57-negative molecules of each DQ beta chain showed different degrees of susceptibility and resistance, susceptibility is not exclusively determined by the DQA1 and DQB1 genes. The combined results of comparisons of homodimers among DR, DQA1 and DQB1 might be more appropriate in terms of finding the result of the total summation of linkage disequilibria. Moreover, we should consider the fact that the susceptibility and the protective effect of the HLA class II genes in T1DM are determined by the competition between the binding affinities of each HLA molecule and pancreatic beta cell antigens [43].

There is also an ethnic difference in the pattern of these disequilibria. In Caucasians, a disequilibrium is formed in DR4 as DQA1*0301-DQB1-0302, and in Japanese, as DQA1*0301-DQB1*0401 [44]. Comparing the heterodimer formed from DQA1 and DQB1, which is possible in both cis and trans locations, in the child-onset patient group, those carrying DQA1*0301 with DQB1*0201 and 0303, and those carrying DQA10501 with DQB1*0201, 0302 and 0303 had a higher risk of developing DM. DQA1*0501-DQB1*0303 had the highest OR of 3.4, higher than those of each factor. Thus, we might be tempted to believe that this form of heterodimer

is a risk factor for DM, but possibly due to the lack of adult-onset patients carrying risk factors on DQA1 and DQB1, the risk was not increased, compared to the control. In addition, when we pursued homodimer related to a specific DR, in the child-onset patient group, in DR3, DQA1*0301-DQB1-0201 and DQA1*0501-DQB1*0201, in DR4, DQA1*0301-DQB1*0201, and in DR9, DQA1*0301-DQB1*0303 were increased. However, in the adult-onset patient group, no DQ heterodimer was found to be related to a specific DR. It is possible that there was no dominant DQ molecule in the adult-onset patient group, and that there is a possibility of forming a disequilibrium between a susceptible DR molecule and defensive DQ molecule or between a susceptible DQ molecule and defensive DR molecule.

There has been a continuous controversy over the increased expression of autoimmune antibody in a group with a specific HLA antigen already known to be a high risk factor. Those carrying the heterodimer of HLA-DR had a higher positive rate of ICA and GADA [33, 45], and patients with HLA-DQ showed a higher positive rate of GADA and IA-2A than those without [30]. In particular, patients with DQA1*0301-DQB1*0302 had a higher titre of IA-2A, while those with onset after the age of 10 showed no such correlation [46]. This might imply an association between genetic factors and autoimmune antibody [47]. In addition, in a Japanese study, when patients with DR4-DQ4 were compared to those with DR9-DQ9, the residual C-peptide function was found to reduce more rapidly in the latter group, and patients with longer than 2 years of duration in the DR9-DQ9 group expressed higher GADA titres. These results suggest that the DR9-DQ9 genotype might induce the helper T lymphocyte function against beta-cells, or their destruction via autoimmunity [48].

In our study, when we compared the positive rates of GADA, taking DQA1-DQB1 heterodimer, which occurred at high frequency in the child-onset patient group, as a standard, the heterodimer consisting of DQA1 and AQB1 showed a high positive rate of GADA. In particular, in the group with DQA1*0501-DQB1*0201 heterodimer, there was a significantly higher positive rate. However, in the adult-onset patient group, without any specific high-risk heterodimer, no differences in the positive rates of autoantibody were observed. Yet, when we analysed on the basis of homodimer disequilibrium using DR4 and DR9, compared to the DR4-DQ4 group, the DR9-DQ9 group showed a higher positive rate of autoantibody. To confirm an association between HLA and GADA, further research is needed.

In conclusion, in adult-onset T1DM in Koreans, the positive rate of GADA was found to be similar to that of Caucasians, suggesting considerable involvement of autoimmunity in the pathogenesis of DM, and this result was similar in child-onset patients. However, unlike the high incidence of HLA DR3 and DR4 in the child-onset T1DM patient group, there was a high incidence of HLA-

DR3, -DR4 and -DR9 in the adult-onset T1DM patients, though in terms of the DQ genotype, the distribution was similar. We attribute the high frequency of DR9 in Japanese and Korean T1DM patients to regional characteristics. Also, by measuring GADA titre in adult-onset (after 20 years old) diabetes patients with ambiguous type of DM, physicians are able to predict the presence of insulin dependency in early stages and use this information in treatment plans.

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