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Clinical and genetic characteristics of patients with autoimmune thyroid disease with anti-islet autoimmunity

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Abstract

In contrast to the large number of studies on autoimmunity against the thyroid gland in patients with type 1 diabetes mellitus, little is known about the anti-islet autoimmune status in patients with autoimmune thyroid diseases (AITDs). We therefore studied the anti-islet autoimmune status in patients with AITD and the clinical and genetic characteristics of AITD patients with anti-islet autoimmunity. The positivity and titer of glutamic acid decarboxylase antibody (GAD Ab) were studied in 866 Japanese patients with AITD (546 with Graves disease and 320 with Hashimoto thyroiditis), 221 patients with thyroid disease of nonautoimmune origin, and 282 control subjects. The clinical characteristics and genotypes of *HLA-DRB1*, *DQB1*, and *CTLA4* were compared between AITD patients with and without GAD Ab. The prevalence of GAD Ab was significantly higher in AITD patients than in control subjects (5.8% vs 2.1%, $P = .01$), particularly in Graves disease (7.1% vs 2.1%, $P = .0019$). The prevalence of diabetes mellitus was significantly higher in AITD patients with GAD Ab than in those without (40.0% vs 10.1%, $P < .0001$), particularly in those with a high titer of GAD Ab (high vs low titer: 64% vs 16%, $P = .001$) and also in those positive for insulinoma-associated antigen 2 (IA-2) Ab (IA-2 positive vs negative: 75.0% vs 31.3%, $P = .016$). The AITD patients with GAD Ab were characterized by younger age at onset of diabetes, lower body mass index, higher hemoglobin A_{1c} level, and higher frequency of insulin therapy than those without GAD Ab. The frequency of the *DRB1*0405-DQB1*0401* haplotype was significantly higher in AITD patients with GAD Ab than in those without GAD Ab and control subjects. A single nucleotide polymorphism (rs3087243) of *CTLA4* was significantly associated with AITD, but not with positivity of GAD Ab. These results indicate that patients with AITD, and in particular Graves disease, are prone to develop β-cell autoimmunity and insulin-requiring diabetes, particularly those with a high titer of GAD Ab and/or positive for both GAD and IA-2 Ab. Glutamic acid decarboxylase Ab positivity in AITD patients was associated with HLA, conferring susceptibility to type 1 diabetes mellitus.

1. Introduction

Both type 1 diabetes mellitus and autoimmune thyroid diseases (AITDs), including Graves disease and Hashimoto thyroiditis, are organ-specific autoimmune diseases affecting insulin-producing β-cells of the pancreas and the thyroid gland, respectively. Both are multifactorial diseases caused by a complex interaction of genetic and environmental factors, with genetic factors consisting of multiple susceptibility genes. Among susceptibility genes, HLA and CTLA4 polymorphisms have been reported to be associated with type 1 diabetes mellitus as well as AITD [1-9].

Patients with type 1 diabetes mellitus frequently develop other organ-specific autoimmune diseases, of which AITD is the most frequent disorder [10-12]. Type 1 diabetes mellitus patients complicated by AITD show some differences in clinical and genetic characteristics from those without AITD. Clinically, type 1 diabetes mellitus patients with AITD have been reported to have glutamic acid decarboxylase autoantibodies (GAD Abs) for a longer time and at higher titers

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than patients without AITD [11]. Genetically, the association with *CTLA4* was reported to be concentrated in patients with type 1 diabetes mellitus complicated by AITD [9]. In contrast to the large number of studies on autoimmunity against the thyroid gland in patients with type 1 diabetes mellitus, little is known about the anti-islet autoimmune status in patients with AITD. We therefore studied the anti-islet autoimmune status in patients with AITD, and the clinical and genetic characteristics of AITD patients with anti-islet autoimmunity were compared with those of patients without.

2. Methods

2.1. Subjects

A total of 866 Japanese patients with AITD (695 female and 171 male; age [mean ± SD], 50.2 ± 16.3 years; body mass index [BMI], 21.7 ± 3.3 kg/m^2) were studied. The AITD patients consisted of 546 patients with Graves disease (422 female and, 124 male; age, 45.8 ± 15.6 years; BMI, 21.2 ± 3.1 kg/m^2) and 320 patients with Hashimoto thyroiditis (273 female and 47 male; age, 57.8 ± 14.5 years; BMI, 22.7 ± 3.5 kg/m^2). Autoimmune thyroid diseases was diagnosed clinically by endocrinologists and confirmed by abnormal levels of thyroid hormones and autoantibodies to thyrotropin receptor, thyroid peroxidase, and/or thyroglobulin. As a control group, 221 patients (age, 57.1 ± 16.0 years; BMI, 21.8 ± 3.2 kg/m^2) with thyroid disease of nonautoimmune origin such as subacute thyroiditis or thyroid nodules and 282 healthy control subjects (age, 52.4 ± 14.4 years; BMI, 22.6 ± 3.3 kg/m^2) who underwent annual health checkup were also studied. The serum thyrotropin level and status of thyroid autoantibodies were not investigated in control subjects. Positivity of GAD Ab and its titer in patients with AITD were compared with those in patients without thyroid autoimmunity. The status of diabetes mellitus and other clinical characteristics as well as the genetic characteristics in patients with AITD positive for GAD Ab were compared with those in patients negative for GAD Ab. This study was approved by the appropriate ethical committees, and informed consent was obtained from all participants.

2.2. Methods

2.2.1. Autoantibody assay

Glutamic acid decarboxylase Ab was measured by a commercially available radioimmunoassay kit using ^{125}I-labeled recombinant human GAD65 as a tracer reagent (Cosmic, Tokyo, Japan) [13]. Samples were defined as GAD Ab positive when the level was higher than a threshold of 1.5 U/mL as suggested by the manufacturer [14,15]. This assay had a sensitivity of 82% and specificity of 92% in the first Proficiency Test of Diabetes Autoantibody Standardization Programs organized by the Immunology of Diabetes Society [16].

Autoantibody to insulinoma-associated antigen 2 (IA-2 Ab) was measured by an immunoprecipitation assay using ^{125}I-labeled IA-2 [17]. Samples were defined as IA-2 Ab positive when the level was higher than 1.0 U/mL [17].

2.2.2. Genotyping of HLA-DR, -DQ, and CTLA4

The *HLA-DRB1* and *-DQB1* alleles were genotyped in all AITD patients positive for GAD Ab (n = 42) as well as in age- and sex-matched AITD patients negative for GAD Ab (n = 158). *DRB1* and *DQB1* data from healthy subjects in our previous study (n = 230) [8] served as controls. *DRB1* and *DQB1* were genotyped by polymerase chain reaction–restriction fragment length polymorphism and polymerase chain reaction–sequence-specific oligonucleotide (SSO) and/or sequence-based typing (SBT) methods as described previously [8,18-20]. Haplotypes were determined based on the most probable haplotypes according to the linkage disequilibria in the Japanese population [21,22].

A single nucleotide polymorphism (SNP) in the CTLA-4 gene, rs3087243 (+6230G>A), which has been reported to be associated with type 1 diabetes mellitus as well as AITD [6,7,9], was genotyped in 189 patients with AITD as reported previously [9]. Genotype data of healthy subjects in our previous study served as control [9].

2.2.3. Statistical analysis

χ^2 test and Fisher exact probably test were used to determine the significance of differences in the distribution of the number of subjects and alleles. Student *t* test was used to compare the levels of clinical parameters. Statistical significance was defined as $P < .05$.

3. Results

3.1. Prevalence and clinical characteristics of AITD patients positive for GAD Ab

The prevalence of positivity for GAD Ab in AITD patients was significantly higher than that in healthy control subjects (5.8% vs 2.1%, $P = .01$) (Table 1). The prevalence in patients with Graves disease was significantly higher than that in control subjects (7.1% vs 2.1%, $P = .0019$) as well as in patients with Hashimoto thyroiditis (7.1% vs 3.4%, $P = .02$) (Table 1). The prevalence in patients with Hashimoto thyroiditis was slightly, but not significantly, higher than that in healthy control subjects (3.4% vs 2.1%, not significant [NS]). To confirm the positivity of GAD Ab, 42 AITD

Table 1
Frequency of subjects positive for GAD antibodies

			GAD Ab (+) n (%)	P[a] (vs control)
AITD	Total	(n = 866)	50 (5.8%)	.01
	Graves disease	(n = 546)	39 (7.1%)	.0019
	Hashimoto thyroiditis	(n = 320)	11 (3.4%)	NS
Controls		(n = 282)	6 (2.1%)	

[a] χ^2 test.

patients positive for GAD Ab were repeatedly tested for GAD Ab. All but 2 patients positive for GAD Ab at the first test were positive for GAD Ab at the second test (Supplementary Figure 1). The 2 patients who became negative for GAD Ab at the second test had a low titer (1.6 and 1.5 U/mL) of GAD Ab at the first test (Supplementary Figure 1), suggesting the importance of the titer in studying the prevalence of GAD positivity.

To compare the titer of GAD Ab in patients with AITD with that in other conditions, patients with thyroid diseases of nonautoimmune origin (n = 221) were tested for GAD Ab. Although the prevalence of GAD Ab was not significantly different between AITD patients and patients with thyroid diseases of nonautoimmune origin (5.8% vs 4.5%), the titer of GAD Ab was markedly higher in AITD than in other conditions (Fig. 1). None of the patients with thyroid diseases of nonautoimmune origin as well as healthy control subjects showed a titer higher than 10 U/mL, whereas 23 (46%) of 50 AITD patients positive for GAD Ab showed a titer higher than 10 U/mL ($P = .017$).

The prevalence of diabetes mellitus was significantly higher in AITD patients positive for GAD Ab than in those negative for GAD Ab (40.0% vs 10.1%, $P < .0001$). When AITD patients positive for GAD Ab were divided into 2 groups according to the titer of GAD Ab, a high-titer group and a low-titer group, so that each group contained approximately the same number of subjects, the prevalence of diabetes mellitus was significantly higher in the high-titer group than in the low-titer group (64% vs 16%, $P = .001$). When the clinical characteristics of AITD patients with diabetes mellitus were compared between those with and without GAD Ab, age at onset of diabetes mellitus was significantly younger, BMI was significantly lower, and hemoglobin A_{1c} (HbA_{1c}) level and the frequency of patients treated with insulin were significantly higher in patients with GAD Ab than in those without (Table 2).

To study the positivity of other islet-related autoantibodies, we measured IA-2 Ab in patients who were

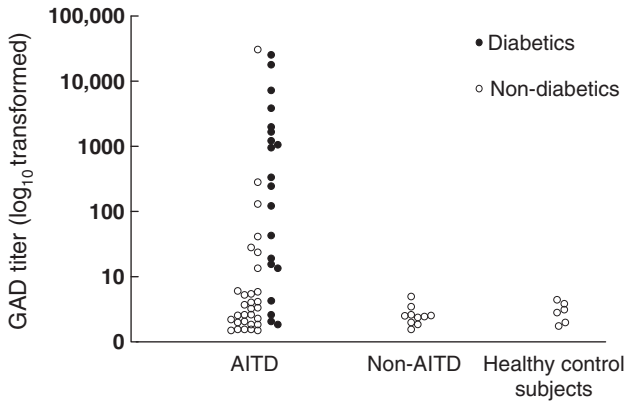

Fig. 1. Titer of GAD antibodies in antibody-positive patients with AITD, patients with thyroid diseases of nonautoimmune origin, and healthy control subjects. Closed circles, patients with diabetes; open circles, nondiabetic patients.

Table 2
Clinical characteristics of AITD patients with diabetes mellitus relative to positivity of GAD antibodies

	GAD Ab (+) (n = 20)	GAD Ab (−) (n = 83)	*P*
GAD Ab titer (mean U/mL) (range)	3176.8 (1.9-26100)		
Graves disease/Hashimoto thyroiditis	17/3	42/41	.006
Age at onset of diabetes (y)	43.2 ± 10.2	53.3 ± 14.2	.004
BMI (kg/m^2)	20.4 ± 3.8	24.1 ± 4.8	.002
HbA_{1c} (%)	8.8 ± 2.3	7.0 ± 1.3	<.0001
Treatment with insulin	85.0%	14.4%	<.0001

Fisher exact probability test for number of patients and Student *t* test for clinical parameters.

positive for GAD Ab. Among 44 patients with GAD Ab, 12 patients (27.3%) were also positive for IA-2 Ab. When positivity of IA-2 Ab was studied relative to the titer of GAD Ab, all 12 patients with IA-2 Ab had a high titer of GAD Ab (mean, 1439; range, 15.7-7310 U/mL). The prevalence of diabetes was significantly higher in patients with both GAD and IA-2 Ab than in patients with GAD Ab alone (75.0% vs 31.2%, $P = .016$).

To study the impact of age on positivity of islet-related autoantibodies, AITD patients who were younger than 30 years (n = 99) were compared with those 30 years or older (n = 767). The prevalence of GAD Ab in younger patients was not significantly different from that in older patients (4.0% vs 6.0%, NS).

3.2. Genetic background

The frequencies of the *DRB1*0405* and *DQB1*0401* alleles and *DRB1*0405-DQB1*0401* haplotype were significantly higher in AITD patients with GAD Ab than in those without GAD Ab and control subjects (Table 3, Supplemental Tables 1 and 2). When HLA in AITD patients positive for GAD Ab was compared between those with and without diabetes, the frequencies of haplotypes known to confer susceptibility to type 1 diabetes mellitus in Japanese, *DRB1*0405-DQB1*0401* and *DRB1*0901-DQB1*0303*, tended to be higher and those of haplotypes known to provide protection against type 1 diabetes mellitus in Japanese, *DRB1*1501-DQB1*0602* and *DRB1*1502-DQB1*0601*, tended to be lower in diabetic patients than in nondiabetic patients (Table 4). The frequencies of genotypes with 2 doses of susceptible haplotypes (*DRB1*0405-DQB1*0401* and *DRB1*0901- DQB1*0303*) were significantly higher in diabetic patients than in nondiabetic patients (52.6% vs 17.4%, $P < .05$). The frequencies of genotypes with at least 1 dose of resistant haplotypes (*DRB1*1501-DQB1*0602* or *DRB1*1502-DQB1*0601*) tended to be lower in diabetic patients than in nondiabetic patients (5.3% vs 30.4%, $P = .05$) (Table 4).

The frequencies of the *DRB1*0803* allele and *DRB1*0803-DQB1*0601* haplotype were significantly

Table 3
Frequency of *DRB1-DQB1* haplotype in patients with AITD with and without GAD Ab and control subjects

DRB1-DQB1	AITD		Control (n = 230)c	*P* (*Pc*[a])		
	GAD (+) (n = 42)	GAD (−) (n = 158)		GAD (+) vs GAD (−)	GAD (+) vs control	GAD (−) vs control
0101-0501	1 (2.4)	8 (5.1)	37 (16.1)	NS	.01 (NS)	.0009 (.013)
0403-0302	1 (2.4)	11 (7.0)	9 (3.9)	NS	NS	NS
0405-0401	25 (59.5)	50 (31.6)	67 (29.1)	.0009 (.013)	.0001 (.0014)	NS
0406-0302	0 (0)	11 (7.0)	14 (6.1)	NS	NS	NS
0410-0402	3 (7.1)	8 (5.1)	6 (2.6)	NS	NS	NS
0802-0302	1 (2.4)	6 (3.8)	4 (1.7)	NS	NS	NS
0802-0402	4 (9.5)	3 (1.9)	9 (3.9)	.04 (NS)	NS	NS
0803-0601	7 (16.7)	44 (27.8)	32 (13.9)	NS	NS	.0007 (.0098)
0901-0303	14 (33.3)	31 (19.6)	59 (25.7)	NS	NS	NS
1101-0301	0 (0.0)	13 (8.2)	11 (4.8)	NS	NS	NS
1201-0301	1 (2.4)	9 (5.7)	16 (7.0)	NS	NS	NS
1302-0604	1 (2.4)	13 (8.2)	27 (11.7)	NS	NS	NS
1501-0602	4 (9.5)	26 (16.5)	20 (8.7)	NS	NS	.02 (NS)
1502-0601	4 (9.5)	21 (13.3)	54 (23.5)	NS	.04 (NS)	.01 (NS)
Others[b]	9 (21.4)	46 (29.1)	75 (32.6)			

Data are number (percentage) of subjects.
[a] *P* value corrected for number of haplotypes tested (n = 14).
[b] Haplotypes with frequency <5% in each group.

higher in AITD patients negative for GAD Ab, but not in those positive for GAD Ab, than in control subjects (Table 3, Supplemental Tables 1 and 2). When GAD Ab–positive patients with and without diabetes were compared, the frequency of the *DRB1*0803-DQB1*0601* haplotype was significantly higher in nondiabetic patients (30.4%) than in diabetic patients (30.4% vs 0.0%, *P* = .009) (Table 4). The frequency of the *DRB1*0101-DQB1*0501* haplotype was significantly lower in AITD patients negative for GAD Ab than in control subjects.

The SNP rs3087243 (+6230G>A) of *CTLA4* was significantly associated with AITD. The frequency of the G allele was significantly higher in AITD patients than in control subjects (odds ratio, 1.41; 95% confidence interval, 1.07-1.87; *P* = .016) (Supplemental Table 3). No significant difference was observed in the frequency of the *CTLA4* genotype between AITD patients with and without GAD Ab (odds ratio, 1.12; 95% confidence interval, 0.58-2.19; NS).

4. Discussion

The present study demonstrated that the prevalence of anti-islet autoimmunity as assessed by GAD Ab was significantly higher in patients with AITD than in healthy control subjects. The AITD patients with GAD Ab showed a significantly higher frequency (40% vs 10%) of diabetes than those without GAD Ab; and this was more pronounced

Table 4
Frequencies of *DRB1-DQB1* haplotypes and genotypic combinations of haplotypes in AITD patients relative to GAD Ab and diabetes status

DRB1-DQB1	GAD (+)		GAD (−) (n = 158) (C)	Control (n = 230) (D)	*P* value			
	DM (+) (n = 19) (A)	DM (−) (n = 23) (B)			A vs B	A vs C	A vs D	B vs D
Haplotypes								
0405-0401	13 (68.4)	12 (52.2)	50 (31.6)	67 (29.1)	NS	.002	.0004	.023
0802-0302	0 (0.0)	1 (4.3)	6 (3.8)	4 (1.7)	NS	NS	NS	NS
0803-0601	0 (0.0)	7 (30.4)	44 (27.8)	32 (13.9)	.009	.008	NS	.036
0901-0303	9 (47.4)	5 (21.7)	31 (19.6)	59 (25.7)	NS	.01	NS	NS
1501-0602	0 (0)	4 (17.4)	26 (16.5)	20 (8.7)	NS	NS	NS	NS
1502-0601	1 (5.3)	3 (13.0)	21 (13.3)	54 (23.5)	NS	NS	NS	NS
Genotypes								
S/S	10 (52.6)	4 (17.4)	13 (8.2)	18 (7.8)	.02	<.0001	<.0001	NS
S/X	7 (36.8)	5 (21.7)	52 (32.9)	76 (33.0)	NS	NS	NS	NS
X/X	1 (5.3)	3 (13.0)	47 (29.7)	64 (27.8)	NS	.03	.03	NS
P/Y	1 (5.3)	7 (30.4)	46 (29.1)	72 (31.3)	.05	.03	.02	

Data are number (percentage) of subjects. S indicates haplotypes that confer susceptibility to type 1 diabetes mellitus, *DRB1*0405-DQB1*0401* and *DRB1*0901-DQB1*0303*; P, haplotypes that provide protection against type 1 diabetes mellitus, *DRB1*1501 -DQB1*0602* and *DRB1*1502-DQB1*0601*; X, haplotypes other than S or P; Y, any haplotype.

in patients with a high titer of GAD Ab, as shown by the prevalence of diabetes of 64% in the high-titer group as compared with 16% in the low-titer group. Diabetes in AITD patients with GAD Ab was characterized by younger age at onset, lower BMI, higher HbA_{1c}, and higher frequency of insulin treatment than that in patients without GAD Ab, suggesting that diabetes in AITD patients positive for GAD Ab shows the clinical features of type 1 diabetes mellitus.

The prevalence of GAD Ab in patients with AITD was 5.8%, which was significantly higher than that in healthy control participants in this study (2.1%) as well as the previously reported prevalence in subjects with normal glucose tolerance (0.6%) [23]. In particular, the prevalence of GAD Ab in patients with Graves disease was much higher than that in control subjects (7.1% vs 2.1%, $P < .0019$) and was similar to the prevalence reported for Graves disease in previous studies [24-26]. The prevalence of GAD Ab in Hashimoto thyroiditis, on the other hand, was slightly, but not significantly, higher than that in control subjects, indicating that the increased prevalence of GAD Ab in AITD in the present study was mostly due to its increase in Graves disease.

In addition to positivity, the importance of the titer of GAD Ab was indicated by the marked difference in titer; but the prevalence of GAD Ab positivity was similar in AITD patients and patients with thyroid diseases of nonautoimmune origin in the present study (Fig. 1). None of the patients with thyroid diseases of nonautoimmune origin positive for GAD Ab showed a titer higher than 10 U/mL, whereas 23 (46%) of 50 AITD patients positive for GAD Ab showed a titer higher than 10 U/mL. None of the GAD-positive patients with thyroid diseases of nonautoimmune origin developed diabetes, whereas 40% of GAD-positive AITD patients developed diabetes. Even within AITD patients, the frequency of diabetes correlated with the titer of GAD Ab, with a 4-fold higher prevalence in the high-titer group than in the low-titer group. The titer in healthy control subjects positive for GAD Ab was also less than 10 U/mL (Fig. 1). A high titer of GAD Ab (>10 U/mL) was previously reported to be a marker for activated T-cell response to β-cell destruction and a high risk for progression to insulin dependence in adult-onset patients with diabetes mellitus [14,27,28]. These data indicate the importance of the titer in addition to positivity of GAD Ab in evaluating anti-islet autoimmunity and β-cell destruction in patients with AITD, as in the case of patients with adult-onset diabetes mellitus.

In addition to a high titer of GAD Ab, positivity for multiple islet-related autoantibodies has been reported to more strongly predict insulin requirement in adult diabetic patients [27]. Among AITD patients positive for GAD Ab in the present study, 27.3% were also positive for IA-2 Ab. The frequency of diabetes was significantly higher in patients with both GAD and IA-2 Ab than in those with GAD Ab alone. All AITD patients positive for both GAD Ab and IA-2 Ab had a high titer of GAD Ab (>10 U/mL). These data suggest that AITD patients with a high titer of GAD Ab and/or patients positive for multiple autoantibodies are at risk for the development of diabetes. Because the present study is a cross-sectional study, prospective studies on diabetes and β-cell function in AITD patients positive for GAD Ab are necessary to further clarify whether or not nondiabetic AITD patients positive for GAD Ab develop diabetes and progress to insulin deficiency.

Genetic analysis of HLA in AITD patients with GAD Ab showed high frequencies of haplotypes known to confer susceptibility to type 1 diabetes mellitus and low frequencies of haplotypes known to provide protection against type 1 diabetes mellitus, indicating that AITD patients positive for GAD Ab differed genetically from those negative for GAD Ab, showing HLA genotypes typical of those in type 1 diabetes mellitus. These data, together with the clinical characteristics of patients with AITD positive for GAD Ab, suggest that AITD patients with GAD Ab have the genetic and clinical characteristics of type 1 diabetes mellitus.

The *DRB1*0803-DQB1*0601* haplotype was associated with AITD without GAD Ab, but not with AITD with GAD Ab, suggesting that the *DRB1*0803-DQB1*0601* haplotype confers susceptibility to autoimmunity against the thyroid gland, but not anti-islet autoimmunity. The association of the *DRB1*0803-DQB1*0601* haplotype with Graves disease has previously been reported in Japanese [4,18] and Korean populations [29]. In contrast, the *DRB1*0405-DQB1*0401* haplotype, which confers susceptibility to type 1 diabetes mellitus, was associated with AITD positive for GAD Ab, but not with AITD negative for GAD Ab, suggesting that the *DRB1*0405-DQB1*0401* haplotype is associated with anti-islet autoimmunity in subjects with as well as without AITD.

In addition to HLA, *CTLA4* has been reported to be associated with both AITD and type 1 diabetes mellitus. Previous studies showed that the association of *CTLA4* with type 1 diabetes mellitus was concentrated in patients complicated by AITD [9]. In fact, it was suggested that the weak association of *CTLA4* with type 1 diabetes mellitus may be secondary to the strong association of *CTLA4* with AITD and the high frequency of AITD complicated by type 1 diabetes mellitus. In the present study, *CTLA4* was associated with AITD as a whole; but no association with GAD Ab positivity was observed in AITD patients. These data further support the possibility that *CTLA4* is primarily associated with AITD; but the contribution of *CTLA4* to anti-islet autoimmunity and type 1 diabetes mellitus is weak, if any.

In conclusion, the present study demonstrated that the prevalence of GAD Ab was high, 5.8%, in AITD patients, and in Graves disease in particular (7.1%), and that 40% of these patients were diabetic, with clinical and genetic characteristics suggestive of type 1 diabetes mellitus, suggesting that AITD patients are prone to develop β-cell autoimmunity and insulin-requiring diabetes, in particular in those with a high titer of GAD Ab and/or positive for both GAD and IA-2 Ab. Prospective follow-up studies in nondiabetic patients with AITD positive for GAD Ab are

necessary to clarify the factors contributing to the development of diabetes, β-cell destruction, and insulin deficiency.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2010.07.025.

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