Clinical and genetic characteristics of autoimmune polyglandular syndrome type 3 variant in the Japanese population

Abbreviated title: Clinical and genetic characteristics of APS3v

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Abstract

Type 1 diabetes (T1D) is commonly associated with autoimmune thyroid disease (AITD), and the occurrence of both T1D and AITD in a patient is defined as autoimmune polyglandular syndrome type 3 variant (APS3v). We aimed to clarify the differences in the clinical and genetic characteristics of APS3v patients and T1D patients without AITD (T1D/AITD(-)) in the Japanese population. Our subjects were 54 APS3v patients and 143 T1D/AITD(-) patients who were consecutively diagnosed at Nagasaki University Hospital from 1983 to the present. A remarkable female predominance, a slow and older age onset of T1D and a higher prevalence of glutamic acid decarboxylase autoantibodies were observed in APS3v patients compared to T1D/AITD(-) patients. The older onset age of T1D in APS3v patients was associated with a higher proportion of slow-onset T1D. Among the two major susceptible HLA class II haplotypes in Japanese T1D, DRB1*0405-DQB1*0401, but not DRB1*0901-DQB1*0303, was associated with APS3v patients. Furthermore, DRB1*0803-DQB1*0601 was not protective in patients with APS3v. The frequencies of the GG genotype in +49G>A and +6230G>A polymorphism in the CTLA4 gene were significantly higher in T1D/AITD(-) patients, but not in APS3v patients, compared to control subjects. In conclusion, we found notable differences in the clinical and genetic characteristics of APS3v patients and T1D/AITD(-) patients in the Japanese population, and the differences in the clinical characteristics between the two groups may reflect distinct genetic backgrounds including the HLA DRB1-DQB1 haplotypes and CTLA4 gene polymorphisms.

INTRODUCTION

Type 1 diabetes (T1D) is caused by the autoimmune destruction of pancreatic β cells. T1D is commonly associated with other organ-specific autoimmune disorders, including autoimmune thyroid disease (AITD), Addison disease, autoimmune gastritis, pernicious anemia, celiac disease, vitiligo and myasthenia gravis (1-6).

It is known that AITD is the most common (>90%) organ-specific autoimmune disease that occurs as a complication in T1D patients in Japan (7, 8). Because of the different genetic background from that of Caucasians, it is known that organ-specific autoimmune diseases consisting of autoimmune polyglandular syndrome type 2 or type 3 together with T1D such as Addison disease, celiac disease or vitiligo are very rare in Japan. The occurrence of AITD in patients with T1D is referred to as a variant of autoimmune polyglandular syndrome type 3 (APS3v) (1, 9-11). It has been shown that APS3v tends to cluster in certain families, and various studies have found that several genes including the HLA, cytotoxic T lymphocyte antigen 4 (CTLA4) and protein tyrosine phosphatase non-receptor type 22 (PTPN22) genes are associated with APS3v across different ethnic groups including Caucasians, Japanese, Koreans and Chinese (1, 9-12).

Despite the large number of genetic and epidemiological studies on APS3v, there has been little investigation of the clinical characteristics of APS3v, such as the female-to-male ratio, or the mean age at the onset of T1D and accompanying AITD, focusing especially on differences compared to T1D patients without AITD. We therefore particularly examined the clinical characteristics of T1D patients with and without AITD, and also reassess genetic backgrounds.

MATERIALS AND METHODS

Disease definition

The diagnosis of T1D is based on the criteria and classification of the Japan Diabetes Society (13). T1D is characterized by destructive lesions of pancreatic β cells either by an

autoimmune mechanism or of unknown cause. For the purposes of the present study, patients who were not proven to have any anti-islet autoantibody positivity (idiopathic T1D) were excluded. APS3v is defined as the development of AITD either before, simultaneous with or after the onset of T1D in a patient. AITD includes Graves' disease (GD) and Hashimoto thyroiditis (HT), which were diagnosed clinically by endocrinologists based on the diagnostic criteria of the Japan Thyroid Association (14). GD was defined as a history of primary hyperthyroidism with positive thyroid-stimulating hormone (TSH) receptor autoantibodies, and HT was defined as having diffuse goiter and/or primary hypothyroidism with positive autoantibodies to thyroid peroxidase and/or thyroglobulin. Patients who were positive only for anti-thyroid autoantibodies without a definitive medical record of thyroid dysfunction or goiter formation were not defined as HT. T1D patients without AITD (T1D/AITD(-)) were defined as having no history of GD, having thyroid dysfunction, and testing negative for both thyroid peroxidase autoantibodies (TPOAbs) and thyroglobulin autoantibodies (TgAbs).

T1D patients, with or without AITD, were divided into two groups according to the mode of diabetes onset, specifically abrupt-onset or slow-onset (15). Abrupt-onset meets the following criteria: 1) the presence of ketosis or ketoacidosis at the onset of diabetes; 2) the presence of hyperglycemic symptoms for less than 3 months before the commencement of insulin therapy; 3) required insulin replacement therapy at both onset and 6 months after onset; 4) the presence of at least one anti-islet autoantibodies (glutamic acid decarboxylase autoantibodies (GADAbs), insulinoma-associated antigen-2 autoantibodies (IA-2Abs), insulin autoantibodies (IAA), or zinc transporter 8 autoantibodies (ZnT8Abs)). Slow-onset T1D meets the following criteria: 1) originally diagnosed as type 2 diabetes and no sign of ketosis at diabetes onset; 2) proven anti-islet autoantibody positivity; 3) insulin treatment started later than 6 months after diagnosis. T1D patients without a medical record of the mode of diabetes onset were excluded from this study.

Patients

We identified 302 Japanese subjects with autoimmune T1D diagnosed at Nagasaki University Hospital from 1983 to the present. These patients were consecutively recruited to avoid selection bias. Of these 302 patients, 105 were excluded from this study for the reasons described above: 14 patients without a medical record of their mode of T1D onset, 73 with positive TPOAbs and/or TgAbs but without a medical record of thyroid dysfunction or goiter formation, and 18 without any data on AITD. Thus, our subjects were 197 patients with T1D, including 54 with APS3v (30 GD and 24 HT) and 143 with T1D/AITD(-). Informed consent was obtained from all subjects included in this study, which was approved by the Ethics Committee of the Nagasaki University.

Autoantibody assay

GADAbs, TgAbs and TPOAbs were measured using a commercially available radioimmunoassay (RIA) kit (Cosmic Corporation, Tokyo, Japan). IAA and TSH receptor autoantibodies (TRAbs) were measured using an RIA kit provided by Yamasa Corporation (Chiba, Japan). IA-2Abs were measured using an RIA kit (Cosmic) and/or a radioligand binding assay (RBA) as previously described (16). ZnT8Abs were measured by RBA as previously described (17).

Genotyping of HLA and non-HLA genes

The HLA-DRB1 and -DQB1 alleles were genotyped as reported previously by Kawabata et al. (18). HLA data were available for 47 patients with APS3v and 101 T1D/AITD(-) patients. Genomic DNA obtained from 222 unrelated healthy individuals served as a control group.

We also genotyped two single-nucleotide polymorphisms (SNPs) in the CTLA4 gene: +49G>A (rs231775) and +6230G>A (rs3087243) (19); one SNP in the promoter of the PTPN22 gene: -1123G>C (rs2488457) (20); and two SNPs in the interleukin-2 receptor-α (IL-2RA) gene, also known as CD25: rs706778 and rs3118470 (21).

Statistical analysis

The results are given as a mean ± SD, unless otherwise indicated. Statistical analysis was performed with the Chi-square test and Student's t test. The significance of differences in the distribution of the HLA DRB1-DQB1 haplotype and non-HLA gene polymorphism was determined by Chi-square test. The odds ratio and its 95% confidence interval (CI) were also calculated. A P value of less than 0.05 was considered statistically significant.

RESULTS

Female-to-male ratio

The patients with autoimmune T1D included in the present study (N=197) consisted of 76 males and 121 females (M:F=1:1.59), showing a mild female predominance. There was also a slight female predominance in patients with T1D/AITD(-) with a female-to-male ratio of 1.17. In contrast, in APS3v patients, there were 4.4 times as many female patients as males and thus the female predominance was obvious. The difference between the female-to-male ratios of APS3v patients and T1D/AITD(-) patients was statistically significant ($p<0.001$, Table 1).

Onset of diabetes

As shown in Table 1 and Fig. 1, the mean age at the onset of diabetes in APS3v patients (33.5±16.9 years) was significantly older than that in patients with T1D/AITD(-) (23.6±17.5 years) ($p<0.001$). It is known that T1D develops in an abrupt- or slow-onset fashion (see *MATERIALS AND METHODS* above for their definitions). Of the 197 patients studied, 129 (65.5%) were classified as abrupt-onset and 68 patients (34.5%) as slow-onset, showing the predominance of the abrupt-onset type. However, when we analyzed patients with APS3v and those with T1D/AITD(-) separately, abrupt-onset diabetes was even more common (74.8%) in T1D/AITD(-) subjects compared to APS3v subjects (40.7%, $p<0.0001$). There was no difference in the mean age at onset of abrupt-onset diabetes or that of slow-onset diabetes

between APS3v and T1D/AITD(-) patients (Table 1 and Fig. 1).

Anti-islet autoantibodies

It is known that titers of anti-islet autoantibodies decrease and often disappear within several years according to increasing duration of diabetes. Thus, the data of anti-islet autoantibodies were analyzed where data had been obtained less than 3 years after the onset of diabetes (30 of 54 patients with APS3v and 74 of 143 patients with T1D/AITD(-)) (Fig. 2). The prevalence of anti-islet autoantibodies in T1D patients overall was 71.3% for GADAbs, 53.1% for IA-2Abs, and 38.7% for ZnT8Abs. When we analyzed APS3v and T1D/AITD(-) subjects separately, the prevalence of GADAbs was significantly higher in APS3v patients than in patients with T1D/AITD(-) (93.1% vs. 62.5%, $p<0.01$). There was no significant difference in the prevalence of GADAbs between abrupt-onset and slow-onset patients (Fig. 2). Although the prevalence of IA-2Abs was not statistically different between APS3v patients and patients with T1D/AITD(-), it was notable in APS3v patients only that the prevalence of IA-2Abs was significantly higher in abrupt-onset diabetes than in slow-onset diabetes (76.9% vs. 26.7%, $p<0.01$). Furthermore, the prevalence of ZnT8Abs was higher in APS3v patients than in patients with T1D/AITD(-) (53.8% vs. 32.8%, $p=0.062$). Higher prevalence of ZnT8Abs in APS3v patients was also observed in both abrupt-onset (63.6% vs. 37.0%, $p=0.10$) and slow-onset (46.7% vs. 15.4%, $p=0.077$) patients (Fig. 2). These results indicate that the repertoire of anti-islet autoantibodies detected in T1D patients is influenced by the presence of AITD and mode of diabetes onset. The prevalence and levels of IAA were not studied because few sera from patients untreated with insulin therapy were stored.

Further analysis of APS3v patients

We then divided the APS3v patients into two groups, T1D with GD (T1D+GD) and T1D with HT (T1D+HT), and examined the clinical features of each group (Table 1). There were no significant differences in the female-to-male ratio, mode of diabetes onset, age at diabetes onset

or prevalence of each anti-islet autoantibody between the two groups. Since it was impossible to determine the age at onset of HT, we focused on the 30 patients with T1D+GD and studied the chronological order of T1D and GD. Sixty percent of patients developed GD before the onset of T1D and 30% developed GD after the onset of T1D; there were also a few patients who developed T1D and GD simultaneously (10%) (Table 1, Fig. 3). The interval between the onsets of T1D and GD was less than 10 years in most cases, but close to 20 years or more than 20 years in some cases (Fig. 3). A comparison of clinical characteristics revealed that slow-onset diabetes was more common in APS3v patients who developed GD before T1D than in those developed GD after T1D (72.2% vs. 33.3%, p=0.053), suggesting that the presence of GD may influence the speed of β-cell destruction (Table 1).

The frequencies of the HLA DRB1-DQB1 haplotype and non-HLA gene polymorphisms

Since we found distinct clinical features between patients with APS3v and those with T1D/AITD(-), we examined whether or not these two subtypes have different genetic backgrounds. We focused on the HLA DRB1-DQB1 haplotype (Table 2) and non-HLA gene polymorphisms (Table 3), all of which have been shown to be associated with T1D.

The HLA DRB1*0405-DQB1*0401 haplotype was significantly more frequent both in patients with APS3v and in patients with T1D/AITD(-) compared to healthy control subjects. However, the DRB1*0901-DQB1*0303 haplotype was significantly more frequent only in patients with T1D/AITD(-) compared to controls. Additionally, two major protective haplotypes in Japanese patients with T1D, DRB1*1501-DQB1*0602 and DRB1*1502-DQB1*0601, were significantly less frequent in both patient groups. The frequency of the DRB1*0803-DQB1*0601 haplotype was significantly lower in T1D/AITD(-) patients, but not in APS3v patients, compared to controls.

SNPs in the CTLA4 (19, 22, 23), PTPN22 (20, 22) and IL2RA genes (21) which were reported previously as being associated with T1D were also examined. The frequencies of the

GG genotype in +49G>A and +6230G>A polymorphism in the CTLA4 gene were significantly higher in overall T1D patients (OR=1.56, $p<0.05$ and OR=1.63, $p<0.05$) and T1D/AITD(-) patients (OR=1.92, $p<0.01$), but not in APS3v patients, than control subjects (Table 3 and Supplementary Table 1). However, the associations tended to be stronger in APS3v patients compared to T1D/AITD(-) patients among the subjects with T1D onset <30 years. Furthermore, these CTLA4 SNPs were associated with abrupt-onset diabetes (Supplementary Table 1). There were no significant differences in other SNPs among APS3v patients or T1D/AITD(-) patients compared to controls (Table 3).

DISCUSSION

This study demonstrates several differences in the clinical features of APS3v patients and T1D/AITD(-) patients.

The sex ratio was remarkably different between APS3v patients and T1D/AITD(-) patients. The female-to-male ratio of overall T1D patients (n=302), including those with T1D for whom we have no data regarding their AITD status, was approximately 1.62 in this study. This ratio was almost equal to or showed slightly higher female predominance than those in epidemiological studies of Japanese who developed T1D at less than 15 years of age (24, 25) and before the age of 30 (26). However, in the present study, female predominance was observed only in patients with APS3v, and not in patients with T1D/AITD(-). These results lead to the implication that patients with APS3v are more classical as an “autoimmune” entity. This is consistent with the higher prevalence of females in all estimates of autoimmune diseases such as GD, HT, rheumatoid arthritis, SLE etc. (27). This female predominance, 4.4 times as many females as males, was more obvious than those reported previously by us (28, 29) and others (23, 30), which ranged from 1.4 to 2.5. The higher female-to-male ratio in patients with APS3v in the present study may possibly be explained by the different definition of HT. Patients with anti-thyroid autoantibodies were included in the HT group whether or not the patient was reported to have had a diffuse goiter or primary hypothyroidism in previous reports (23, 28-30).

In fact, the female predominance became milder by tentatively adding 73 T1D patients who were positive for anti-thyroid autoantibodies without a medical record of thyroid dysfunction and/or goiter formation to APS3v patients group in our study (female-to-male ratio 2.6, data not shown).

In contrast to countries with a low incidence of T1D including Japan (24-26, 31-33), it is known that, in other countries with a high incidence of T1D, the incidence of T1D is higher in males than in females. Our findings suggest that the female predominance of T1D in our country might be due to the higher frequency of APS3v in patients with T1D because AITD is known to be developed more commonly in females.

We also found a difference in the mode of T1D onset between patients with APS3v and those with T1D/AITD(-). Our data demonstrated for the first time that slow-onset T1D is more frequent than abrupt-onset T1D in APS3v patients, while 75% of T1D/AITD(-) patients have the abrupt-onset form. Since the development of slow-onset T1D occurs at an older age than that of abrupt-onset T1D, the difference in the high frequency of slow-onset T1D seems to influence the mean age at the onset of diabetes: an older onset of diabetes in APS3v patients and a younger onset in T1D/AITD(-) patients.

We found that the repertoire of anti-islet autoantibodies is associated not only with the presence of AITD but also with the mode of diabetes onset. GADAbs were more frequently detected in patients with APS3v, which is consistent when the mode of T1D onset is taken into consideration. The prevalence of IA-2Abs in patients with abrupt-onset diabetes was higher than that in patients with slow-onset diabetes in patients with APS3v but not in patients with T1D/AITD(-). Additionally, to the best of our knowledge, the present study is the first to show that the frequency of ZnT8Abs is higher in patients with APS3v than in patients with T1D/AITD(-) (53.8% vs. 32.8%, p=0.062). However, this difference did not reach statistical significance, likely due to small number of APS3v patients. The distinct repertoires of anti-islet autoantibodies observed in APS3v and T1D/AITD(-) patients might reflect the distinct pathogenesis between these two subtypes.

It has been previously reported that there are differences in the HLA haplotype between patients with APS3v and patients with T1D/AITD(-) in both Caucasians (1, 3, 5, 10, 11) and Japanese (29, 30, 34), and these studies show that the susceptible HLA haplotypes are dependent on race. We showed that DRB1*0405-DQB1*0401 is a common susceptible haplotype in patients with APS3v and T1D/AITD(-). However, the prevalence of the DRB1*0901-DQB1*0303 haplotype was significantly higher only in T1D/AITD(-) patients. Since we showed that more patients with T1D/AITD(-) develop abrupt-onset T1D, these findings are compatible with the previous study by Kawabata et al., who found that the DRB1*0901-DQB1*0303 haplotype is associated with abrupt-onset T1D (35). The DRB1*0803-DQB1*0601 haplotype has also been described to be a protective haplotype for abrupt-onset T1D in Japanese (35), so it is consistent that the frequency of the DRB1*0803-DQB1*0601 haplotype was significantly lower in T1D/AITD(-) patients.

It has been shown that the GG genotype or G allele of +49G>A and +6230G>A polymorphism in the CTLA4 gene are associated with T1D patients with anti-thyroid autoimmunity among Japanese (19, 22, 23), which appears to be the opposite of the present findings. In contrast, it has also been reported that the GG genotype of +49G>A polymorphism is not associated with T1D patients with AITD among Japanese (36). This seems to be explained by the different definition of HT (19, 22, 23) and different choice of subjects, i.e., only those who developed T1D before the age of 30 (23). The GG genotype of +49G>A and +6230G>A polymorphism also tends to be higher than healthy controls in T1D patients with anti-thyroid autoimmunity, including those with only anti-thyroid autoantibodies (OR=1.26 and OR=1.28). Moreover, by taking up only the patients who developed T1D under 30 years of age, the frequency of the GG genotype of +49G>A and +6230G>A polymorphism in APS3v patients tends to be higher than that in T1D/AITD(-) patients (OR=2.13 vs. 1.92 and OR=2.70 vs. 2.07). Furthermore, and interestingly, the GG genotype of +49G>A and +6230G>A polymorphism was associated with abrupt-onset diabetes (OR=2.10, $p<0.01$ for +49G>A and OR=1.93, $p<0.05$ for +6230G>A), indicating that CTLA4 polymorphism may be one of the factors influencing

the mode of T1D onset (Supplemental Table 1). This is consistent with our previous report that the G allele frequency of +49G>A polymorphism of the CTLA4 gene in abrupt-onset T1D patients was significantly higher than that in type 2 diabetes GADAb-positive patients (37), and with another report that the GG genotype of +49G>A polymorphism significant increase in younger-onset (<30 years) T1D but not in older-onset (≥30 years) T1D because the ratio of abrupt-onset T1D is estimated to be higher in the younger-onset T1D group (23).

In conclusion, we demonstrated that the contribution of AITD to the gender difference, mode of diabetes onset, and anti-islet humoral autoimmunity in patients with T1D, which may be associated with a distinct genetic background including the class II HLA and CTLA4 gene polymorphism. These findings include some novel findings concerning the clinical and genetic features of APS3v and might be a key to understanding the pathogenesis of both T1D and APS3v.

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FIGURE LEGENDS

Figure 1. The onset of T1D in APS3v patients and T1D/AITD(-) patients. Open and closed circles represent the onset of diabetes in abrupt-onset and slow-onset T1D patients, respectively. Data are shown for individual patients. Data are expressed as means ± SD. *, $P < 0.001$; **, $P < 0.000000001$.

Figure 2. The prevalence of anti-islet autoantibodies in APS3v patients and T1D/AITD(-) patients. Closed and open bars represent the prevalence of GADAbs, IA-2Abs and ZnT8Abs in APS3v and T1D/AITD(-) patients, respectively. *, $P < 0.01$.

Figure 3. The interval from the onset of T1D to the onset of GD in APS3v patients. The open, closed and shaded bars represent the individual patients who developed GD after T1D, GD and T1D simultaneously, and GD before T1D.

Fig.1

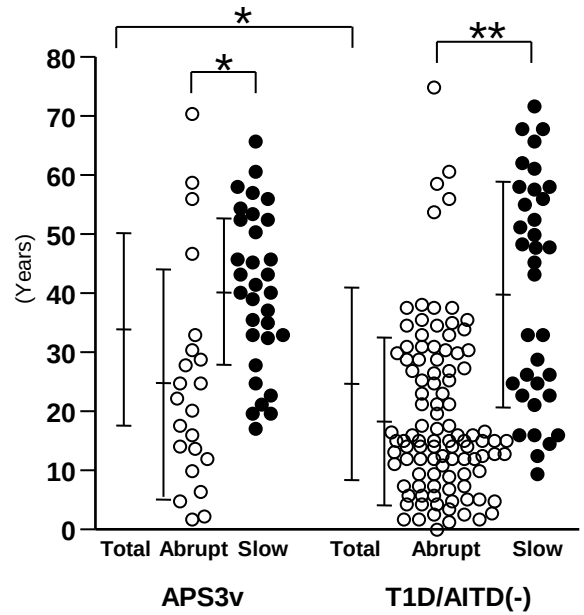
*
**
*
80
70
60
50
40
30
20
10
0
(Years)
Total
Abrupt
Slow
Total
Abrupt
Slow
APS3v
T1D/AITD(-)

Fig.2

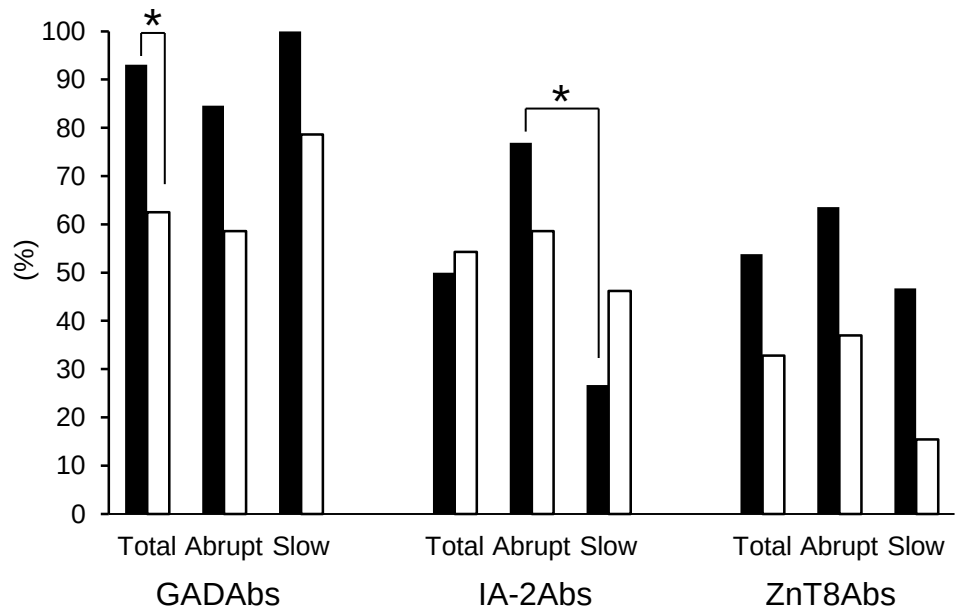
100
90
80
70
60
50
40
30
20
10
0
(%)
*
*
Total Abrupt Slow
Total Abrupt Slow
Total Abrupt Slow
GADAbs
IA-2Abs
ZnT8Abs

Fig.3

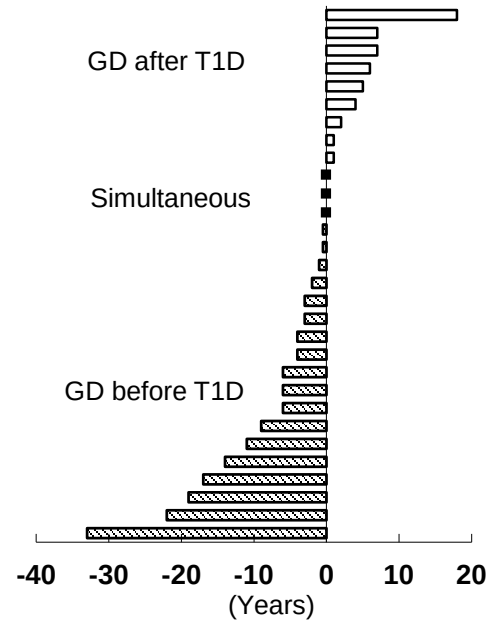
GD after T1D
Simultaneous
GD before T1D
-40
-30
-20
-10
0
10
20
(Years)

Table 1 Clinical characteristics of APS3v and T1D/AITD(-)

T1D with or without AITD	**APS3v** (n=54)	**T1D/AITD(-)** (n=143)	P value
Sex (Male/Female)	10/44	66/77	<0.001
Mode of T1D onset (Abrupt/Slow)	22/32	107/36	<0.00001
Age at onset of T1D (Total subjects)	33.5±16.9	23.6±17.5	<0.001
(Abrupt T1D)	24.7±18.3	18.5±13.6	NS
(Slow T1D)	39.6±12.9	39.0±19.0	NS
P value (Abrupt vs. Slow)	<0.001	<0.000000001	

APS3v with GD or HT	**with GD** (n=30)	**with HT** (n=24)	P value
Sex (Male/Female)	6/24	4/20	NS
Mode of T1D onset (Abrupt/Slow)	12/18	10/14	NS
Timing of AITD onset (before T1D/ simultaneous/after T1D)	18/3/9	ND	
Age at onset of T1D (Total subjects)	36.1±15.7	30.3±18.0	NS
(Abrupt T1D)	26.8±18.3	22.2±19.0	NS
(Slow T1D)	42.4±10.2	36.1±15.4	NS
P value (Abrupt vs. Slow)	<0.05	NS	
Age at onset of AITD (Total subjects)	32.5±15.5	ND	
(Abrupt T1D)	27.9±17.0	ND	
(Slow T1D)	35.6±14.1	ND	
P value (Abrupt vs. Slow)	NS		
Interval between T1D and AITD	7.0±7.9	ND	

APS3v with GD	**GD before T1D** (n=18)	**Simultaneous** (n=3)	**GD after T1D** (n=9)	P value (bef. vs. aft.)
Sex (Male/Female)	3/15	1/2	2/7	NS
T1D onset (Abrupt/Slow)	5/13	1/2	6/3	NS
Age at onset of T1D	37.9±13.8	36.3±13.6	32.4±20.6	NS
Age at onset of GD	29.1±13.5	36.3±13.6	38.1±19.3	NS
Interval between T1D and GD	8.8±9.0	0.0	5.7±5.2	NS

Data are n or years (means ± SD). APS3v, autoimmune polyglandular syndrome type 3 variant; T1D/AITD(-), Type 1 diabetes without autoimmune thyroid disease; GD, Graves' disease; HT, Hashimoto thyroiditis; NS, not significant; ND, no data.

Table 2 Frequency of the HLA DRB1-DQB1 haplotype in APS3v, T1D/AITD(-) and healthy controls

	APS3v (n=94)	T1D/AITD(-) (n=202)	Controls (n=444)	APS3v vs. T1D/AITD(-)		APS3v vs. Controls		T1D/AITD(-) vs. Controls	
DRB1-DQB1	n (%)	n (%)	n (%)	P value	OR (95%CI)	P value	OR (95%CI)	P value	OR (95%CI)
*0101-*0501	2 (2.1)	6 (3.0)	25 (5.6)	NS		NS		NS	
*0301-*0201	1 (1.1)	7 (3.5)	4 (0.9)	NS		NS		NS	
*0403-*0302	3 (3.2)	0 (0.0)	10 (2.3)	NS		NS		NS	
*0405-*0401	26 (27.7)	60 (29.7)	58 (13.1)	NS		<0.001	2.54 (1.52-4.27)	<0.000001	2.81 (1.89-4.19)
*0406-*0302	4 (4.3)	2 (1.0)	16 (3.6)	NS		NS		NS	
*0802-*0302	4 (4.3)	7 (3.5)	9 (2.0)	NS		NS		NS	
*0803-*0601	8 (8.5)	4 (2.0)	35 (7.9)	<0.01	4.60 (1.49-14.2)	NS		<0.01	0.24 (0.09-0.62)
*0901-*0303	24 (25.5)	69 (34.2)	83 (18.7)	NS		NS		<0.0001	2.26 (1.56-3.27)
*1302-*0604	8 (8.5)	17 (8.4)	27 (6.1)	NS		NS		NS	
*1501-*0602	0 (0.0)	3 (1.5)	28 (6.3)	NS		<0.05	0.00 (0.00-0.00)	<0.01	0.22 (0.07-0.68)
*1502-*0601	3 (3.2)	11 (5.4)	51 (11.5)	NS		<0.05	0.25 (0.08-0.77)	<0.05	0.44 (0.23-0.86)
Others	11 (11.7)	16 (7.9)	98 (22.1)	NS		<0.05	0.47 (0.24-0.90)	<0.0001	0.30 (0.18-0.52)

“Others” includes rare haplotypes whose total frequencies in each group were less than 3.0%.

OR, odds ratio; NS, not significant.

Table 3 Frequency of non-HLA polymorphism in APS3v, T1D/AITD(-) and healthy controls

			APS3v (n=47)	T1D/AITD(-) (n=98)	Controls (n=222)
CTLA4	**+49G>A**	AA	4 (8.5)	12 (12.2)	42 (18.9)
		AG	27 (57.4)	37 (37.8)	104 (46.8)
		GG	16 (34.0)	49 (50.0)	76 (34.2)
		OR[a] (vs CTRL)	0.99	1.92	-----
		95%CI	0.51-1.93	1.19-3.11	-----
		P value	NS	<0.01	-----
CTLA4	**+6230G>A**	AA	1 (2.1)	6 (6.1)	18 (8.1)
		AG	18 (38.3)	29 (29.6)	91 (41.0)
		GG	28 (59.6)	63 (64.3)	113 (50.9)
		OR[b] (vs CTRL)	1.42	1.74	-----
		95%CI	0.75-2.69	1.07-2.83	-----
		P value	NS	<0.05	-----
PTPN22	**-1123G>C**	CC	16 (34.0)	30 (30.6)	80 (36.0)
		CG	20 (42.6)	45 (45.9)	103 (46.4)
		GG	11 (23.4)	23 (23.5)	39 (17.6)
		OR[c] (vs CTRL)	0.86	0.98	-----
		95%CI	0.45-1.62	0.61-1.58	-----
		P value	NS	NS	-----
IL-2RA	**rs706778A>G**	AA	13 (27.7)	33 (33.7)	73 (32.9)
		AG	28 (59.6)	43 (43.9)	109 (49.1)
		GG	6 (12.8)	22 (22.4)	40 (18.0)
		OR[d] (vs CTRL)	0.78	1.04	-----
		95%CI	0.39-1.57	0.63-1.72	-----
		P value	NS	NS	-----
IL-2RA	**rs3118470A>G**	AA	9 (19.1)	25 (25.5)	50 (22.5)
		AG	29 (61.7)	49 (50.0)	109 (49.1)
		GG	9 (19.1)	24 (24.5)	63 (28.4)
		OR[e] (vs CTRL)	0.60	0.82	-----
		95%CI	0.27-1.30	0.47-1.41	-----
		P value	NS	NS	-----

Data are n (%). [a, b, e] For the GG genotype. [c] For the CG genotype. [d] For the AA genotype.

OR, odds ratio; NS, not significant; CTRL, Controls.