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SHORT COMMUNICATION

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Association of the T-cell regulatory gene *CTLA4* with Graves' disease and autoimmune thyroid disease in the Japanese

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Abstract Autoimmune thyroid disease (AITD) is caused by an immune response to self-thyroid antigen. The cytotoxic T-lymphocyte antigen-4 (*CTLA4*) gene, encoding a negative regulator of the T-lymphocyte immune response, had been reported to be associated and/or linked to AITD. Recently, AITD susceptibility in the Caucasians was mapped to the 6.1-kb 3′UTR of the *CTLA4* gene, in which the three single-nucleotide polymorphisms (SNPs) CT60, JO31, and JO30 were strongly associated with AITD. In order to determine the association of the *CTLA4* gene with AITD in the Japanese, case-control association analysis for the four SNPs of the *CTLA4* gene using 380 AITD patients and 266 healthy controls was done. Among the SNPs examined, the SNP JO31 was most significantly associated with AITD in the Japanese, whereas the association of the JO30 with AITD was not observed. The frequency of the disease-susceptible G allele of the JO31 of the Japanese control was higher than that of the Caucasians (67.1% vs 50.2%); however, the G allele of the JO31 was associated with Graves' disease (GD) (67.1% vs 76.3%, $P = 0.0013$) and AITD in the Japanese (67.1% vs 74.2%, $P = 0.0055$). Furthermore, the G allele of the JO31 was associated with the increased risk for GD [$P = 0.0051$, odds ratio (OR) = 1.7] and AITD ($P = 0.016$, OR = 1.5) in a dominant model. These results suggested that the *CTLA4* gene is involved in the susceptibility for GD and AITD in the Japanese.

Keywords Autoimmune thyroid disease (AITD) · Graves' disease · CTLA4 · Single-nucleotide polymorphism (SNP) · Association

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Introduction

The autoimmune thyroid disease (AITD), including Graves' disease (GD) and Hashimoto's thyroiditis (HT), is caused by multiple genetic and environmental factors (Stassi and De Maria 2002). In addition to a strong contribution to susceptibility by the*HLA* locus (Uno et al. 1981; Dong et al. 1992; Wan et al. 1995), GD and HT have been reported to be associated and/or linked to the *CTLA4* locus (Yanagawa et al. 1995; Donner et al. 1997; Kotsa et al. 1997; Heward et al. 1999; Vaidya et al. 1999a, b). However, the causal polymorphism(s) at this locus remained to be elucidated. Recently, AITD susceptibility in the Caucasians was mapped to the 6.1-kb 3′UTR of the *CTLA4* gene, in which the SNP CT60 was most significantly associated with GD ($P = 1.6\times10^{-6}$) and HT ($P = 0.0005$) (Ueda et al. 2003). This allelic variation was reported to be correlated with lower mRNA levels of the soluble alternative splice form of *CTLA4* (*sCTLA4*) (Ueda et al. 2003). Previously, we carried out a genome-wide screening using the non-parametric sib-pair method in 123 Japanese sib-pairs affected with AITD, in which the multipoint maximum LOD score for AITD at the marker *D2S117* near *CTLA4* was 0.52, indicating no evidence of linkage for the *CTLA4* locus (Sakai et al. 2001). However, linkage analysis has low power of detection to identify loci with relatively small genetic effects, whereas case-control association study has strong advantages for the

Table 1 Association of the SNPs in the *CTLA4* gene with autoimmune thyroid disease (AITD) in the Japanese (data from Ueda et al. 2003)

Maker/allele	Case (%)	Control (%)	*P*-value	Odds ratio	95% CI
+49/G	65.0 (42.4)	60.3 (35.8)	0.13 (0.0021)	1.2 (1.34)	0.9–1.6 (1.11–1.62)
CT60/G	78.0 (63.4)	72.0 (52.3)	0.016 (1.6×10^{-6})	1.4 (1.56)	1.1–1.8 (1.30–1.88)
JO31/G	74.2 (61.0)	67.1 (50.2)	0.0055 (4.1×10^{-6})	1.4 (1.54)	1.1–1.8 (1.28–1.85)
JO30/G	63.7 (61.5)	61.5 (50.5)	0.48 (1.9×10^{-6})	1.1 (1.56)	0.8–1.4 (1.29–1.87)

Table 2 Association of the SNP JO31 with GD and AITD in the Japanese. *HT* Hashimoto's thyroiditis, *GD* Graves's disease

JO31	Disease	Control	*P*-value	Odds ratio (95% CI)
HT				
Number of individuals analyzed	$n=173$	$n=266$		
Total number of alleles			GG vs GT + TT (recessive model)	
G	251 (72.5%)	357 (67.1%)	0.16	1.6 (0.8–3.3)
T	95 (27.5%)	175 (32.9%)	GG + GT vs TT(dominant model)	
χ^2 (*P*-value)	2.91 (0.087)		0.16	1.3 (0.9–1.9)
GD				
Number of individuals analyzed	$n=235$	$n=266$		
Total number of alleles			GG vs GT + TT (recessive model)	
G	360 (76.3%)	357 (67.1%)	0.017	2.3 (1.1–4.6)
T	112 (23.7%)	175 (32.9%)	GG + GT vs TT (dominant model)	
χ^2 (*P*-value)	10.29 (0.0013)		0.0051	1.7 (1.2–2.4)
AITD				
Number of individuals analyzed	$n=380$	$n=266$		
Total number of alleles			GG vs GT + TT (recessive model)	
G	564 (74.2%)	357 (67.1%)	0.036	1.8 (1.0–3.2)
T	196 (25.8%)	175 (32.9%)	GG + GT vs TT (dominant model)	
χ^2 (*P*-value)	7.72 (0.0055)	0.016	1.5 (1.1–2.0)	

detection of such loci (Risch 1990; Risch and Merikangas 1996). To determine the attribution of *CTLA4* for AITD in the Japanese, we genotyped Japanese AITD patients and healthy controls for the four SNPs of the *CTLA4* gene, including +49, CT60, JO31, and JO30.

Subjects and methods

One hundred and seventy-three HT cases (48 unrelated individuals from the 112 AITD family and 125 unrelated sporadic cases), 235 GD cases (92 unrelated individuals from the 112 AITD family and 143 unrelated sporadic cases), 380 AITD cases (112 unrelated individuals, consisting of 33 HT cases and 79 GD cases from the 112 AITD family, and 268 unrelated sporadic cases), and 266 unrelated healthy control individuals were genotyped. AITD cases were collected from Tokyo, Kyoto, and Kobe, whereas control individuals were from various regions of Japan. These Japanese participants were interviewed and examined and gave written, informed consent. This project was approved by the ethical committee of the related institutes. Diagnosis of thyroid disease was established based on clinical findings and results of routine examinations: circulating thyroid hormone and thyroid stimulating hormone (TSH) concentrations, serum levels of antibodies against thyroglobulin, thyroid microsomes and TSH receptors, ultrasonography, $^{99m}TCO4^-$(or ^{123}I) uptake and thyroid scintigraphy. The patients with atrophic thyroiditis diagnosed by the existence of blocking-type, anti-TSH receptor antibodies were excluded from this study.

Genomic DNA was isolated from peripheral blood cells using QIAamp DNA Blood Midi Kits (Qiagen). The four SNPs in the *CTLA4* gene were genotyped using the TaqMan PCR assay (Applied Biosystems). All reaction was done in a volume of 5 μl containing 5 ng of genomic DNA, following the manufacturer's protocol. SNP genotyping was performed using ABI PRISM 7900HT (Applied Biosystems). Association analyses between controls and cases were carried out using a contingency 2×2 table to calculate an odds ratio (OR) and χ^2 as described. LD mapping (r^2) was done using the EH program through the analysis of the 254 unrelated controls.

Results and discussion

A total of four SNPs (Ueda et al. 2003) were genotyped in 380 cases with AITD and 266 healthy controls. The four SNPs in the *CTLA4* gene examined are as follows: +49 (at position 49 of exon 1) and CT60, JO31, and JO30 in the 3′UTR. Among the four SNPs, CT60 and JO31 were associated with AITD, $P=0.016$ and $P=0.0055$, respectively (Table 1). However, association of the G allele of JO30 with AITD, which SNP showed the strong association with AITD in the Caucasians ($P=1.9\times10^{-6}$), was not observed in the Japanese (Table 1). CT60 and JO31 were in linkage disequilibrium ($r^2=0.8$), while association of the specific haplotype constructed by these two SNPs with AITD was not observed (data not shown), indicating no evidence of haplotype effects for AITD susceptibility.

The SNP CT60 was reported to be most significantly associated with GD and HT in the Caucasians (Ueda et al. 2003). The frequency of the disease-susceptible G allele of the SNP CT60 of the control in the Japanese was much higher than that in the Caucasians (72.0% vs 52.3%); however, the G allele of CT60 was associated with the susceptibility for AITD (72.0% vs 78.0%, $P=0.016$) (Table 1).

In our study, the G allele of the JO31 was most significantly associated with GD ($P = 0.0013$) and AITD ($P = 0.0055$), with the higher allele frequency of the Japanese control than that of the Caucasians (67.1% vs 50.2%) (Tables 1, 2). Furthermore, the G allele of JO31 was associated with the increased risk for GD ($P = 0.0051$, OR $= 1.7$) and AITD ($P = 0.016$, OR $= 1.5$) in a dominant model (Table 2). Interestingly, the G allele of the JO31 in a recessive model was also associated with the increased risk for GD ($P = 0.017$, OR $= 2.3$) and AITD ($P = 0.036$, OR $= 1.8$) (Table 2). These results suggested that the G allele of JO31 may work in a dominant fashion, and that gene dose effect may exist, as the OR in a recessive model was higher than that in a dominant model. Although association of JO31 with HT was not observed in the allele frequency ($P = 0.085$), the G allele of JO31 in HT seemed to be higher than that in control (72.5% vs 67.1%) (Table 2), suggesting that the SNP might be weakly associated with HT. Although the exact functional meanings of these SNPs remain to be elucidated, all these results suggested that the *CTLA4* gene is involved in the susceptibility for GD and AITD in the Japanese.

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