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Association between the TAP1 Gene Codon 637 Polymorphism and Graves' Disease

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A total of 95 patients with Graves' disease (GD) and 105 normal healthy controls were enrolled in this study to determine how a single site polymorphism of the transporter associated with antigen processing 1 (TAP1) gene contributes to the pathogenesis of GD. The polymorphism was detected using polymerase chain reaction (PCR)–based restriction analysis. Associations between GD and the two-site polymorphisms of the TAP1 gene at codons 333 and 637 were evaluated. No significant differences were revealed comparing GD patients and normal individuals for the distributions of genotypes and allelic variants at codon 333 ($p = 0.253$ and $p = 0.891$, respectively). By contrast, the distributions for the AA homozygote at codon 637 were reduced and those for the GA heterozygote were increased comparing the two groups ($p < 0.0001$). The allelic analysis also demonstrated lower A and higher G allele frequencies ($p = 0.0008$; OR = 2.745, 95% CI = 1.482–5.085) comparing the GD patients with the normal healthy controls. This shows that the single-site polymorphism of the TAP1 gene at codon 637 may be an indicator for predicting development of GD.

Key Words: Codon; Graves' disease; polymorphism; transporter associated with antigen processing.

Introduction

Graves' disease (GD), a common autoimmune thyroid disease, is the major cause of hyperthyroidism in patients younger than age 40. Predominantly affecting women, the prevalence rate is around 2%, with reported male:female ratios ranging from 1:10 to 1:7 in caucasoid populations. It is distributed worldwide, and occurs in Asians as common as in whites *(1)*. Clinically, GD is characterized by diffuse goiter, thyrotoxicosis, infiltrative ophthalmopathy, and, occasionally, infiltrative dermopathy.

It is now clear that GD pathogenesis involves intrathyroidal thyrotropin, i.e., thyroid stimulating hormone (TSH), receptor antibodies *(2)*. TSH receptor antibodies (TRAb) are a group of IgG antibodies produced via a chain of immunological processes. Upon stimulation of interferon-γ released from infiltrating T cells, the thyroid cells express human leukocyte antigen (HLA) class II molecules, allowing the cells to present antigens, such as TSH receptors, to the helper T cells. The activated T cells can then facilitate proliferation and differentiation of B cells, with resultant antibody formation *(3)*. Thus, it is clear that the presentation of HLA molecules associated with peptide antigens to the T cells plays a central role in the development of GD.

Transporter associated with antigen processing (TAP) is a multimembrane-spanning protein. It is responsible for translocation of the cytosolic peptide antigens into the endoplasmic reticulum where peptides are loaded onto the newly synthesized HLA class I molecules before they are transported to the plasma membrane and recognized by the cytotoxic T lymphocytes (CTL). TAP is actually a heterodimer, which is composed of two subunits, TAP1 and TAP2. Each subunit consists of two parts, membrane-spanning and cytoplasmic domains. The latter is critical for the selection and binding of antigenic peptides *(4)*. The genes encoding both TAP1 and TAP2 are located within the HLA class II region between the HLA–DP and –DQ loci *(5)*. Hence, it seems reasonable to suggest that the TAP genes are associated with susceptibility to autoimmune disease. In fact, TAP gene polymorphisms have been observed in various diseases, such as atopic dermatitis, type 1 DM, and rheumatoid arthritis *(6–8)*.

Although the results of research into the relationship between TAP1 gene polymorphisms and GD are limited and contradictory, preliminary data have demonstrated that specific allelic variants of the TAP1 gene exist in GD patients of different racial extractions *(9–11)*. To test whether the TAP1 gene could be a genetic marker for prediction of GD development, we screened two polymorphic sites of the TAP1 gene, codons 333 and 637, using polymerase chain reaction (PCR)–based restriction analysis to compare GD patients and normal controls in Taiwan.

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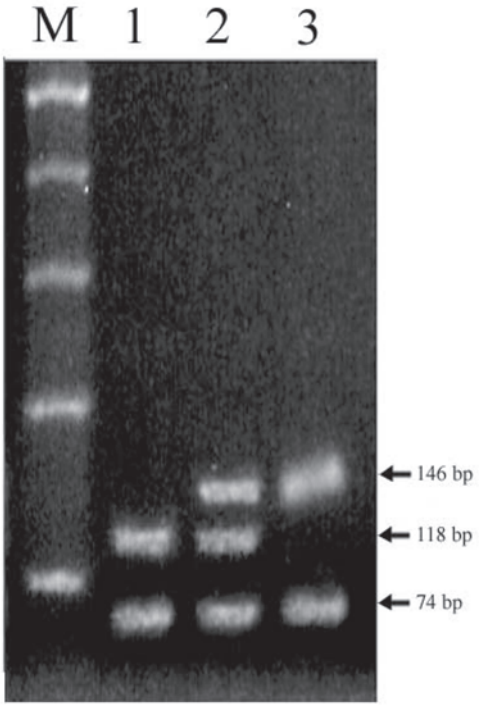

Fig. 1. PCR-based polymorphism analysis of 3% agarose gel electrophoresis. *Dpn*II digestion of the TAP1 gene at codon 333 produced fragements of 118 bp, 74 bp, and 28 bp for Ile (ATC) or 146 bp and 74 bp for Val (GTC). Lanes M, 1, 2, and 3 represent marker, AA homozygote, GA heterozygote, and GG homozygote, respectively.

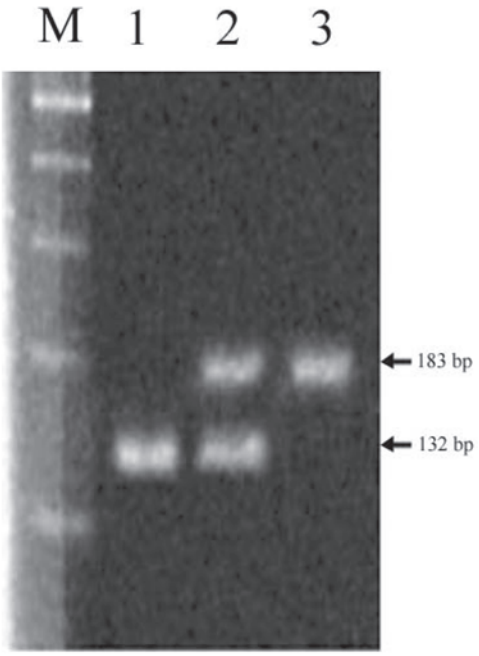

Fig. 2. PCR-based polymorphism analysis of 3% agarose gel electrophoresis. *Acc*I digestion of the TAP1 gene at codon 637 produced fragments of 132 bp and 51 bp for Asp (GAC) or a 183 bp undigested fragment for Gly (GGC). Lanes M, 1, 2 and 3 represent marker, AA homozygote, GA heterozygote, and GG homozygote, respectively.

Results

Figures 1 and 2 show the results of electrophoresis for codons 333 and 637 polymorphisms, respectively. The gel bands revealed excisable (AA) or non-excisable (GG) homozygotes and heterozygotes (GA). The frequencies of the genotypes and allelic variants for the TAP1 gene codon 333 polymorphism in the GD patient and control groups are shown in Table 1. The genotype and allelic frequencies for this site polymorphism were compared using the chi-square test, with no significant differences demonstrated comparing the two groups ($p = 0.253$ and $p = 0.891$, respectively). However, the powers for both insignificances are low (0.34 and 0.05, respectively). By contrast, the distributions for both genotypes and allelic variants of the codon 637 polymorphism were significantly different (Table 2). For genotype distribution, the GD patient group had increased GA and reduced AA frequencies when compared with the control group (34.7% and 63.2% vs 12.4% and 85.7%, respectively; Fisher's exact test, $p < 0.0001$; power = 0.94). Allelic distribution analysis revealed higher G and lower A frequencies relative to the control group (19.5% and 80.5% vs 8.1% and 91.9%, respectively; chi-square test, $p = 0.0008$; power = 0.94). For the G allele, the odds ratio of GD susceptibility was 2.745 (95% CI = 1.482–5.085) comparing with the A allele. In respect of examination for genotype frequencies, polymorphisms of codon 637 as well as codon 333 were found to be in Hardy–Weinberg equilibrium in both control and patient groups.

Discussion

The TAP genes have been implicated as susceptibility genes for GD *(9–11)*. Our study further demonstrates an association between a single-site polymorphism of the TAP1 gene and GD susceptibility. The data reveal increased frequencies for the GA genotype and G allele at codon 637 of the TAP1 gene comparing the GD patients and normal controls. It seems reasonable to suggest, therefore, that the TAP1 gene may be useful as a genetic marker for predicting GD development.

The TAP1 gene polymorphism was first discovered in the early 1990s *(4)*. The major polymorphic sites are codons 333 and 637 (cDNA position 1069 and 1982) *(13,14)*. The relationship between the TAP1 gene polymorphism and GD in causacoid and Chinese populations has been reported separately by Rau et al. *(9)* and by Cai et al. *(11)*. The allelic dimorphic polymorphisms of the TAP1 gene were screened in the two studies, and both groups reported the comparable results, with an increased frequency of the TAP1*0301 allele [Val (GTC)-333/Asp (GAC)-637] and a decreased frequency of the TAP1*0401 allele [Ile (ATC)-333/Gly (GGC)-

Table 1
A Comparison of GD Patients and Normal Controls Stratified According to Frequencies of the Genotypic and Allelic Variants of the TAP1 Gene at Codon 333[a]

	Graves' disease $n = 95$ (%)	Normal controls $n = 105$ (%)	p value[b]
Genotype			0.253
GG	5 (5.3)	10 (9.5)	
GA	40 (42.1)	34 (32.3)	
AA	50 (52.6)	61 (58.2)	
Allelic variant			0.891
G	50 (26.3)	54 (25.7)	
A	140 (73.7)	156 (74.3)	

[a]Results are presented as *n* (%).
[b]Chi-square test.

Table 2
A Comparison of GD Patients and Normal Controls Stratified According to the Frequencies of the Genotypic and Allelic Variants of the TAP1 Gene at Codon 637[a]

	Graves' disease $n = 95$ (%)	Normal controls $n = 105$ (%)	p value
Genotype			<0.0001[b]
GG	2 (2.1)	2 (1.9)	
GA	33 (34.7)	13 (12.4)	
AA	60 (63.2)	90 (85.7)	
Allelic variant[d]			0.0008[c]
G	37 (19.5)	17 (8.1)	
A	153 (80.5)	193 (91.9)	

[a]Results are presented as *n* (%).
[b]Fisher's exact test.
[c]Chi-square test.
[d]For G allele: odds ratio = 2.745 (95% confidence interval = 1.482–5.085).

637] comparing the GD patients with the normal controls. In addition, Rau et al. also observed increased and decreased frequencies for Val-333 and Ile-333 variants in the GD group compared with the control analog. In our study, by contrast, these two-site polymorphisms of the TAP1 gene were screened separately, without dimorphic allelic analysis in both groups. Furthermore, both the genotype distributions and the allelic frequencies of the codon 333 site variants (GTC/ATC) were not statistically different. On the other hand, the frequencies of the GA heterozygote and G allelic variant [GGC (Gly)] were higher and those of the AA homozygote and A allele [GAC (Asp)] were lower for codon 637 comparing the GD group with the controls. The reasons for the discrepancy in the results from different studies are unknown; however, it may reflect genetic differences in the ethnically and geographically distinct study populations.

TAP1 gene codon 637 encodes amino acid located within the cytoplasmic domain *(4,13)*. In theory, regional replacement of aspartic acid by an analog with different chemical and physical properties, glycine, may alter the structure of TAP1 and interfere with its activities for peptide selection and binding. The subsequent peptide transportation and HLA molecule expression must therefore be altered. Regarding the lack of association between codon 333 polymorphism and GD susceptibility in our study, however, the possibility of type II statistical error based on deficiency of power could not be ruled out. Further study with a greater number of samples may be necessary.

The nature of the association between TAP1 gene polymorphism and GD susceptibility is unclear. As most genes within the HLA class II region are in linkage disequilibrium, it is also possible that the TAP1 gene polymorphism contributes to the development of GD through linkage disequilibrium with specific alleles in this region *(15,16)*. Direct, functional activities of the gene polymorphism with respect to the development of GD should not be excluded, however. There are two possible mechanisms. First, the interaction of HLA class I molecules and CTL may be involved in the pathogenesis of GD. Predominantly cytotoxic, but not helper, T cell infiltration in the thyroids of GD patients has been observed by a few authors *(17–19)*. CTL can facilitate the deposition of circulating immune complexes and their local formation and, thereby, play a role in the generation of GD *(17)*. TAP1 is critical for the delivery of peptides and for the presentation of HLA class I molecules to CTL. The TAP1 gene polymorphism may, thus, affect the development of GD via this pathway. Second, the TAP1-mediated pathway may present cytosolic antigens to HLA class II–restricted T cells. Although it is generally considered that TAP is specifically responsible for HLA class I molecule presentation, Malnati et al. have observed that TAP1 is also necessary for HLA class II–restricted presentation of endogenous cytosolic peptides *(20)*. This finding provides additional evidence of the contribution of the TAP1 gene polymorphism to the development of GD, a predominantly HLA class II molecule–mediated autoimmune disease.

Our data indicate that a single site polymorphism of the TAP1 gene, codon 637 [GAC (Asp)/GGC (Gly)], may be a candidate genetic marker for screening for susceptibility to GD in Taiwan. Any subject who bears GA genotype and/or G allelic variant at codon 637 in TAP1 gene should be followed to see whether she (or he) will develop GD.

Patients and Methods

Patient Selection

A total of 95 unrelated Chinese GD patients (21 men and 74 women) aged between 17 and 71 yr (mean 35.3 ± 10.5) were enrolled in the study from July 2003 to November 2003. All the patients were of the Han race and resided in the middle of Taiwan. The presence of hyperthyroidism, diffuse goiter, and positive serum TRAb were used to define GD. The serum antibodies to microsome and thyroglobulin were also tested in all patients and only seven patients (two men and five women) appeared negative in either item or both. The control group consisted of 105 healthy volunteers (22 men and 83 women) over the age of 40 yr who were euthyroid and had no previous personal or family history of hyperthyroidism or any other autoimmune disease. All the healthy controls were also of Han race and resided in the middle of Taiwan. The study was approved by the local ethical committee, and informed consent was obtained from each subject. The genomic DNA was prepared from peripheral blood using the Genomic DNA isolation reagent kit (Genomaker Inc., Taipei, Taiwan).

Polymerase Chain Reaction (PCR)

PCR was used to identify the genotypes of the TAP1 gene codons 333 and 637 for all subjects. Polymerase chain reaction for the codon 333 polymorphism (ATC/GTC) was performed using a total volume of 50 μL containing genomic DNA (2–6 pmole of each primer); 1X Taq polymerase buffer (1.5 m*M* $MgCl_2$); and 0.25 units of AmpliTaq DNA polymerase (Perkin Elmer; Foster City, CA, USA). The primers for this site polymorphism were forward 5'-CACC CTGAGTGATTCTCT-3' and backward 5'-ACTGAGTC TGCCAAGTCT-3', as described by Saiki et al. *(12)*. The PCR for the codon 637 polymorphism (GAC/GGC) was performed using a total volume of 50 μL containing genomic DNA (2–6 pmole of each primer), 1X Taq polymerase buffer (1.5 m*M* $MgCl_2$), and 0.25 units of AmpliTaq DNA polymerase (Perkin Elmer). The primers for this site polymorphism were forward 5'-CCCTATCCAGCTACAACC-3' and backward 5'-AACGCCACTGCCTGTCGCT-3'. PCR amplification was performed in a programmable thermal cycler (GeneAmp PCR System 2400; Perkin Elmer). The cycling conditions for both of the site polymorphisms were set as follows: one cycle at 94°C for 2 min, 35 cycles of 94°C for 15 s, 60°C for 20 s, and 72°C for 20 s, and then one final extension cycle at 72°C for 10 min.

Both site polymorphisms of the TAP1 gene at codons 333 and 637 were discerned by digestion with *Dpn*II and *Acc*I, respectively. The PCR products were mixed separately using the above enzymes and reaction buffer according to the manufacturer's instructions, with both reactions incubated for 3 h at 37°C. Then, 10 μL of each product was loaded into a 3% agarose gel containing ethidium bromide for electrophoresis. The genotypes were classified into excisable allele homozygote (AA) and non-excisable allele homozygote (GG) and heterozygote (GA).

Statistical Analysis

The genotype distributions and allelic frequencies of the polymorphisms for the GD and control groups were statistically compared by the chi-square test using SPSS Version 8.01 (SPSS Inc., Chicago, IL, USA). When the assumption of the chi-square test was violated (i.e., a single cell had an expected count of <1, or, more than 20% of the cells had an expected count of <5), Fisher's exact test was used. Results were considered statistically significant when the probability of findings occurring by chance was less than 5% ($p < 0.05$), which was then further examined with power analysis. Odds ratios (OR) with 95% confidence intervals (CI) were calculated for disease susceptibility associated with specific genotypes and alleles. Finally, the genotype frequencies were examined with the Hardy–Weinberg equilibrium proportions using a chi-square test with one degree of freedom.

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