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TAP1 Gene *Acc*I Polymorphism is Associated With Atopic Bronchial Asthma

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Asthma is a hyperresponsive airway disease that may involve inflammation responses. A transporter associated with the antigen processing 1 gene (TAP1) is involved in antigen processing, and is therefore considered to play a role in the pathogenesis of bronchial asthma. The aim of this study was to test whether the polymorphisms of the TAP1 gene are a genetic marker for susceptibility to bronchial asthma. A normal control group comprised of 43 healthy people, and 116 patients with allergic asthma were examined in this study. The polymorphism was detected by polymerase chain reaction (PCR)-based restriction analysis. Associations between atopic bronchial asthma and TAP1 polymorphisms were evaluated. The results revealed no significant differences between normal individuals and asthmatics in regard to the TAP1 gene *Dpn*II polymorphism (P=0.752). However, there was a significant difference between the control and asthma groups as regards the TAP1 gene *Acc*I polymorphism (P=0.020). The odds ratio (OR) of GG homozygotes of the TAP1 *Acc*I polymorphism was 229.8 compared with the AA homozygote group. The results show that the *Acc*I polymorphism may be an indicator for atopic bronchial asthma. J. Clin. Lab. Anal. 17:57–60, 2003.

Key words: atopic bronchial asthma; single nucleotide polymorphisms (SNPs); transporter and antigen processor gene (TAP)

INTRODUCTION

Asthma is a hyperresponsive airway disease of widely varying severity and multifactorial (environmental and genetic) etiology (1). In a collaborative study on the genetic factors of asthma, researchers reported that chromosomal regions 2q33, 5p15, 11p15, 17q11.1-q11.2, 19q13, and 21q21 are linked to asthma (2). Therefore, appropriate association studies of genetic polymorphisms, a common tool for mapping complex disease genes, may be useful for determining possible genetic markers for asthma (3).

Airway inflammation is a hallmark of asthma. Furthermore, initial inflammation may be caused by the behavior of the adaptive immune system–especially the T helper cell response to allergic stimuli that are inhaled into the airway (4). The interaction between airway epithelial cells and inflammatory cells, such as leukocytes, plays an important role in the immune response (5). The transporter associated with antigen processing 1 (TAP1) genes is a heterodimer molecule. This transporter is responsible for the translocation of peptides. It is located over DQB1 and DRB1, and includes several factors involved in antigen processing related to the MHC class II region. TAP1 essentially participates in the delivery of the peptide to the HLA class I molecule (6). The contribution of TAP1 to the HLA class II restricted endogenous process has also been reported (7). On the other hand, TAP1 may also mediate IFN-γ responsiveness and activate the gene of

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an intercellular adhesion molecule-1 (ICAM-1) that enhances T-cell adhesion and transmigration (8). Because TAP1 is involved in the inflammation process, it could be a potential genetic marker for asthma. To test this hypothesis, we compared the allelic frequencies of TAP1-1 and TAP1-2 polymorphisms in a normal population with those of patients with atopic asthma.

METHODS

Patients and Methods

A control group was drawn up of 43 healthy volunteers with a normal limit of pulmonary function and no history of allergic disease. The patient group consisted of 116 patients (59 males and 57 females, 3–70 years old, average age 22.70 $\pm$ 16.56 years) with a history of bronchial asthma with variable airflow limitation on the disease attack and atopy history. The control group included 13 males and 30 females (16–28 years old, average age 20.79 $\pm$ 2.73 years). The diagnosis of atopy was based on the Pharmacia® CAP system. Informed consent was obtained from all subjects. The genomic DNA was prepared from peripheral blood by use of a genomic DNA isolation reagent kit (Genomaker, Taipei, Taiwan). Polymerase chain reactions (PCRs) for the TAP1-1 *Dpn*II (A/G) were carried out to a total volume of 50 μL, containing genomic DNA (2–6 *p*mole of each primer), 1X Taq polymerase buffer (1.5 mM $MgCl_2$), and 0.25 units of AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA). The primers for the TAP1-1 *Dpn*II (A/G) were forward (5′-CACCCTGAGTGATTCTCT-3′) and backward (5′-ACTGAGTCTGCCAAGTCT-3′) according to the method of Saiki et al. (9). PCR for the TAP1-2 *AccI* (A/G) was carried out to a total volume of 50 μL, containing genomic DNA (2–6 *p*mole of each primer), 1X Taq polymerase buffer (1.5 mM $MgCl_2$), and 0.25 units of AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA). The primers for the TAP1-2 *Acc*I 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 the TAP1 *Dpn*II and *Acc*I (A/G) polymorphisms were set as follows: one cycle at 94°C for 2 min; 35 cycles at 94°C for 15 sec, 60°C for 20 sec, and 72°C for 20 sec; and one final cycle of extension at 72°C for 10 min.

Polymorphisms at TAP1 codon 333 (TAP1-1) and codon 637 (TAP1-2) were discerned by digestion with *Dpn*II and *Acc*I. The PCR product was mixed with the above-mentioned enzymes and the reaction buffer according to the manufacturer's instructions. The restriction site was designed to be located at the allele of TGC to form an excisable site. The reaction was incubated for 3 hr at 37°C. Then, 10 μL of the product were loaded into a 3% agarose gel containing ethidium bromide for electrophoresis. The genotypes were classified as excisable allele homozygote (AA), unexcisable allele homozygote (GG), and heterozygote (AG).

The allelic frequency distribution of this polymorphism between the control and atopic asthma patient groups was compared using the chi-square test. When the assumption of the chi-square test was violated (one cell had an expected count of <1, or $>20\%$ of the cells had an expected count of <5), Fisher's exact test was used. The distributions of the TAP1 *Dpn*II and *Acc*I (A/G) polymorphisms in each group were evaluated. Means and odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for the polymorphisms of the *Dpn*II and *Acc*I (A/G) TAP1 gene.

RESULTS

The frequencies of the genotype in the asthma group and control group are shown in Table 1. Using Fisher's exact test, the distribution of the TAP1 gene *Dpn*II polymorphism was compared. No significant difference between the healthy control group and the asthma patient group was found ($P=0.752$). The allelic distribution in the control and asthma groups was A: 75.58% and 73.85%, and G: 34.42% and 26.15%, respectively. The distribution of the TAP1 gene *Acc*I polymorphism was compared and a significant difference between the healthy control group and the asthma patient group was found ($P=0.020$). The allelic distribution in the control and asthma groups was A: 73.85% and 82.33%, and G: 7.98% and 17.67%, respectively. According to the logistic regression analysis, the GG homozygote carries a risk of developing atopic asthma (OR = 229.8 compared to the AA homozygote) (Table 2).

TABLE 1. The distribution of TAP-1 *Dpn* II and *Acc* I (A/G) polymorphism between healthy control subjects and asthma patients

	AA	A/G	GG	Total	*P* value
Dpn II					
Control	25 (58.1%)	15 (34.9%)	3 (7.0%)	43 (100%)	0.752[a]
Asthma	58 (53.2%)	45 (41.3%)	6 (5.5%)	109 (100%)	
Acc I					
Control	37 (86.0%)	6 (14.0%)	0 (0.0%)	43 (100%)	0.020[b]
Asthma	76 (65.5%)	39 (33.6%)	1 (0.9%)	116 (100%)	

[a]Chi-square test, df = 2, $\chi^2 = 0.570$.
[b]Fisher's exact test.

TABLE 2. Logistic regression analysis of TAP-1 *Acc* I (A/G) polymorphism associated with asthma with adjusted odds ratio

Independent variables	Odds ratio	95% confidence interval	*P* value*
Genotype			
AA	1		
AG	3.2	1.2∼8.1	0.0169
GG	229.8	$0 \sim 2.05 \times 10^{21}$	0.0805
Per copy of the G allele	3.2	1.3∼8.2	0.0140

*$P < 0.05$.

DISCUSSION

The data revealed that the TAP1 gene *Acc*I polymorphism is a good genetic marker for asthma screening. The frequency of the A/G polymorphism distribution was significantly different between asthma patients and the control group. Other possible explanations for the positive results include direct causation, natural selection, population stratification, statistical artifact, and linkage disequilibrium (10). Further investigation is required to determine the association of this polymorphism with asthma.

Asthma is complicated and involves great genetic heterogeneity. They are many linkages of allergy and asthma phenotypes with different regions of the human genome (11). In our clinics, many parents inquire as to whether they have the same genetic risk factors as their children. Several reports have indicated that different HLA products and genes may be protective factors against the development of asthma (12,13). According to our results, the TAP1 gene *Acc*I polymorphism may increase the risk of developing bronchial asthma.

The inhaled antigen from the airway mucosa regulates epithelial–leukocyte interactions, and the epithelial immune response plays an important role in asthma (14). Inhibition of ICAM-1 and chemoattractant expression or function is an attractive target in the therapy of airway inflation (15). TAP1 is one of the most important genes controlling signal transduction between the cell surface and nucleus (16). The TAP1 gene polymorphism may have a different pathway in the immune interaction between the airway epithelium and antigen.

The TAP1 gene polymorphism was discovered in 1993 (16–19) and has been the subject of several studies. By using restriction endonuclease *Dpn*II and *Acc*I to identify a single base difference of the TAP1 gene from individual to individual, we were able to determine the distribution of single nucleotide polymorphisms (SNPs) in the disease group. According to Sooji et al. (18), there is no association between TAP and atopic dermatitis. Their study showed that Tunisians carrying the glycine-637 of the TAP1 protein have an increased risk of atopy, and a specific association between the homozygous TAP1 D/D genotype and allergic asthma (19). By comparing data reported by Awatef et al. (20) with those of the present study, the allelic distribution of TAP-1 gene codons 333 and 637 was determined to be different in Taiwanese of Chinese descent. The results of our study indicate that the TAP1 gene *Acc*I polymorphism may be a useful genetic marker for asthma, in agreement with the data of Awatef et al. (20).

Because of a lack of predictive markers for asthma, the disease is diagnosed in patients after symptoms develop. Therefore, a reliable marker for asthma could aid in earlier diagnosis and treatment, which would significantly improve patient care and help control health-care costs. In conclusion, the present data indicate that the TAP1 gene *Acc*I polymorphism may be a genetic marker for asthma screening.

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