

Distribution of HLA-A, B alleles and polymorphisms of TAP and LMP genes in Korean patients with atopic dermatitis

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Summary

Background Atopic dermatitis has been seen to result from multifactorial inheritance, with interaction between genetic and environmental factors. The genetic association may differ according to the ethnic backgrounds.

Objective The purpose of this study was to investigate the genetic factors in Korean atopic dermatitis patients by studying the human leucocyte antigen (HLA) class I association and polymorphisms of transporters associated with antigen presentation (TAP) and low-molecular-weight polypeptide (LMP) genes.

Methods HLA-A and B genotyping was performed in 53 atopic dermatitis patients and 184 healthy controls using the standard microlymphocytotoxicity technique. TAP1, TAP2, LMP2, and LMP7 gene polymorphisms were analyzed using the polymerase chain reaction (PCR)-single strand conformation polymorphism (SSCP), PCR-amplification refractory mutation system (ARMS), and PCR-restriction fragment length polymorphism (RFLP).

Results Allele frequency of HLA-A24 was significantly increased in patients with atopic dermatitis compared to controls ($P < 0.05$). HLA-B alleles showed no differences in distribution between patients and controls. Genotype, phenotype, and allele frequencies of TAP1 gene also revealed no differences in distribution between patients and controls. Analysis of TAP2 gene polymorphisms showed increased frequencies of the TAP2*C allele and TAP2*A/TAP2*C genotype in atopic dermatitis patients compared to controls ($P < 0.05$). Distribution of LMP2 and LMP7 gene polymorphisms was similar for patients and controls.

Conclusion This study demonstrates an association of atopic dermatitis with HLA-A24 and TAP2*C alleles in Korean patients. Discrepancy with the previous reports might be related to different patient characteristics and ethnic variations.

Keywords: atopic dermatitis, HLA class I, LMP, TAP

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Introduction

There has been a reported increase of atopic dermatitis over the past three decades [1,2] and it appears to show the same trend in Korea [3]. Atopic dermatitis is diagnosed by its clinical and laboratory features, but the exact criteria used for the diagnosis of atopic dermatitis vary widely between investigators. Atopic dermatitis has been regarded as a

result of multifactorial inheritance, with interactions between genetic and environmental factors. Recently, studies on the genetic mechanism of atopy or asthma were performed and an understanding of the genetic basis of atopic dermatitis was suggested to be of importance in managing patients with atopic dermatitis.

Human leucocyte antigen (HLA) molecules are peptide-binding proteins on the surfaces of antigen-presenting cells. The complex of an HLA molecule and bound antigenic peptide form a specific target for T cell recognition. Major histocompatibility complex (MHC) class I molecules

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generally present antigens to CD8⁺ T cells, and class II molecules present antigens to CD4⁺ T cells. Both HLA class I and class II antigens are supposed to contribute to the pathogenesis of atopic dermatitis [4]. In antigen presentation to cytotoxic T cells with HLA class I molecules, the antigen-processing pathway is controlled by the products of genes that are mapped within HLA class I region, low-molecular-weight polypeptide (LMP) and transporters associated with antigen presentation (TAP). TAP genes encode a heterodimer molecule involved in the translocation of intracellular peptides across the endoplasmic reticulum membrane where they bind to class I molecules. Genes encoding the two TAP subunits, TAP1 and TAP2 are located within the MHC class II region between the DPB1 and DQB1 loci [5,6], and variations in rat TAP genes have been reported to be associated with differences in the spectrum of MHC class I-binding peptides [7]. Many diseases associated with HLA have been investigated for the influence of TAP [8–13]. Two dimorphic sites within TAP1, Ile/Val-333 and Asp/Gly-637, and four dimorphic sites within TAP2, Val/Ile-379, Ala/Thr-565, Thr/Ala-665 and Stop/Gln-687, have been widely investigated [9]. LMP2 and LMP7 genes encode two subunits of the proteasome, a cytoplasmic catalytic complex involved in the generation of antigenic peptides that are loaded on class I molecules within the endoplasmic reticulum [14]. LMP2/7 subunits may directly affect peptide cleavage specificity [15,16].

Different HLA genes were reported to be associated with the development of atopic dermatitis [17–20] and asthma [21,22]. Association of TAP gene polymorphism in atopic dermatitis was reported in a study [23], but the association was not found in another study from Japan [4].

The nature of the genetic association may vary according to different ethnic backgrounds. In this study, we investigated the HLA class I association and TAP and LMP gene polymorphisms in Korean patients with atopic dermatitis.

Materials and methods

Subjects

The study included 53 unrelated Korean atopic dermatitis patients (25 females and 28 males; mean age 18.9 ± 9.9 years; age range 5–45 years). All patients had moderate to severe atopic dermatitis according to the criteria of Hanifin and Rajka [24]. After informed consent was obtained, venous blood was drawn from each individual. Skin prick tests were positive in 20/29 (69.0%) tested patients.

Controls consisted of 184 healthy Korean persons without personal or family history of atopic diseases.

HLA class I typing

Serologic typing of HLA-A and B alleles was performed in all patients using the standard lymphocyte microcytotoxicity test [25]. Cells and serum were incubated together for 30 min at 25 °C and incubated at 25 °C for one hour with the addition of complement. Cell death was assessed by adding Eosin-Y.

DNA extraction

Heparinized venous blood 10 mL was drawn from subjects. Genomic DNA was extracted from peripheral blood leucocytes isolated by Ficoll-Hypaque density gradient centrifugation. In brief, lymphocytes (2×10^6 cells/mL) were lysed in 0.5 mL PCR-K buffer ($10 \times$ PCR buffer 1 mL, NP-40 40 µL, Tween-20 45 µL, proteinase K (20 mg/mL) 30 µL, and D/W 8.8 mL) at 58 °C for 60 min, and proteinase K was inactivated at 95 °C for 10 min. The extracted DNA was used as a template in PCR.

PCR-SSCP analysis of TAP gene polymorphisms

Oligonucleotides were synthesized by the DNA synthesizer (Cyclon 8400 plus, Milligen/Bioscience, Milford, MA, USA) according to the method described by Carrington *et al.* [26]. Nomenclature and sequence characteristics of TAP alleles are shown in Table 1. Nucleotide sequences of the primers were described previously [27].

PCR was performed using 1 µL of DNA samples (100 µg/mL) in 10 µL reaction mixtures of Taq polymerase, $10 \times$ buffer (500 mM KCl, 100 mM Tris-HCl pH 9.0, 15 mM MgCl₂, 1% Triton X-100), primers (100 ng/µL), dNTP (10×1 mM dCTP, and 2 mM dATP, dGTP, and dTTP), 32PdCTP (0.7 µCi), and distilled water. PCR conditions were as follows: 95 °C for 30 s for denaturing, 60 °C for 60 s for annealing, and 72 °C for 90 s for primer extension. This cycling was repeated 35 times in a programmable DNA Thermal Cycler (Perkin-Elmer Cetus, Norwalk, CT, USA). Amplified PCR products were subjected to SSCP analysis. In brief, PCR product 3 µL was mixed with 10 µL of stop solution (10 mM NaOH, 95% formamide, 0.05% bromophenol, and 0.05% xylene cyanol) and the reaction mixture was denatured at 95 °C for 2–3 min. The products were separated on a 6% non-denaturing acrylamide gel at 4 °C, 50 watt for 4 h.

PCR-ARMS analysis of TAP2 gene polymorphisms

DNA samples 1 µL (100 µg/mL) were amplified in 10 µL reaction mixtures containing Taq polymerase, $10 \times$ buffer, primers, dNTP (10×2 mM dATP, dGTP, dTTP, and dCTP) and distilled water. PCR cycles consisted of 35 cycles of 30 s denaturation at 95 °C, 60 s annealing at 60 °C,

Table 1. Nomenclature and sequence characteristics of TAP and LMP genes

Amino acid (nucleotide sequence)			
TAP1 alleles	333	637	
TAP1*A	ILE (ATC)	ASP (GAC)	
TAP1*B	VAL (GTC)	GLY (GGC)	
TAP1*C	VAL (GTC)	ASP (GAC)	
TAP2 alleles	379	565	665
TAP2*A	VAL (GTA)	ALA (GCT)	THR (ACA)
TAP2*B	VAL (GTA)	ALA (GCT)	ALA (GCA)
TAP2*C	ILE (ATA)	ALA (GCT)	THR (ACA)
TAP2*D	ILE (ATA)	THR (ACT)	THR (ACA)
TAP2*E	VAL (GTA)	THR (ACT)	THR (ACA)
TAP2*G	ILE (ATA)	ALA (GCT)	ALA (GCA)
LMP2 alleles	60		
LMP2*R	ARG (CGC)		
LMP2*H	HIS (CAC)		
Nucleotide position			
LMP7 alleles	3911	3912	4069
LMP7*A	T	C	C
LMP7*B	G	C	C
LMP7*C	G	C	T
LMP7*D	G	T	T

and 90 s extension at 72 °C. This cycling was performed in a programmable DNA Thermal Cycler (Perkin-Elmer Cetus). Amplified PCR products were separated on a 1.5% agarose gel and stained with ethidium bromide.

PCR-RFLP analysis of LMP gene polymorphisms

Primers for PCR amplification were synthesized using the DNA synthesizer (Cyclon 8400 plus) by the method of Kelly *et al.* [28]. Nucleotide sequences of the primers were as described previously [27]. Nomenclature and sequence characteristics of LMP alleles are shown in Table 1.

DNA samples 2 µL (100 µg/mL) were amplified in 20 µL reaction mixtures containing Taq polymerase, 10 × buffer, primers, dNTP and distilled water. PCR cycles consisted of 35 cycles of 15 s denaturation at 95 °C, 40 s annealing at 67 °C, and 30 s extension at 72 °C. Amplified PCR products were subjected to digestion with a restriction endonuclease, *Hha* I at 37 °C for an hour. After digestion, fragments were fractionated in 8% acrylamide gel at 300 V for 2.5 h and visualized by ethidium bromide staining.

Statistics

HLA-A and B allele frequencies were determined by direct counting. Comparisons between patients and controls were analysed by the two-tailed Fisher's exact probability test

(*P*-value test). *P*-values were corrected (*P_c*) with the number of alleles tested. *P* and *P_c* values of less than 0.05 were considered statistically significant. Relative risk (RR) with 95% confidence interval (CI) was calculated using Woolf's method with Haldane's modification.

Results

Distribution of HLA-A and B alleles in atopic dermatitis patients

Eleven HLA-A (1, 2, 3, 24, 26, 66, 11, 29, 30, 31 and 33) and 27 HLA-B (51, 52, 7, 8, 13, 14, 62, 75, 16, 38, 39, 57, 58, 54, 55, 56, 27, 35, 37, 60, 61, 46, 48, 53, 59, 67 and 71) alleles that are frequent in Koreans were typed. Allele frequency of HLA-A24 was significantly increased in patients with atopic dermatitis (31/53 patients, 58.5%; 73/184 control subjects, 39.7%; *P* < 0.05; RR 2.1426 with 95% CI 1.16–3.96), but the statistical significance was lost after *P*-value was corrected with the number of alleles tested. The other HLA-A and B alleles showed no significant differences between patients and controls.

TAP1 and TAP2 polymorphisms in atopic dermatitis patients

Table 2 summarizes genotype, phenotype, and allele frequencies of TAP1 gene. Distribution of TAP1 gene

Table 2. Distribution of TAP1 alleles in atopic dermatitis patients

Genotype frequencies	Patients (<i>n</i> = 53)		Controls (<i>n</i> = 184)		RR (95% CI)	<i>P</i> -value	<i>P</i> _c -value
	<i>n</i>	(%)	<i>n</i>	(%)			
TAP1*A/TAP1*A	34	(64.2)	113	(61.4)		NS	NS
TAP1*C/TAP1*C	0	(0.0)	1	(0.5)		NS	NS
TAP1*B/TAP1*B	2	(3.8)	4	(2.2)		NS	NS
TAP1*B/TAP1*C	1	(1.9)	6	(3.3)		NS	NS
TAP1*A/TAP1*C	2	(3.8)	11	(6.0)		NS	NS
TAP1*A/TAP1*B	14	(26.4)	49	(26.6)		NS	NS
Phenotype frequencies							
TAP1*A	50	(94.3)	173	(94.0)		NS	NS
TAP1*B	17	(34.0)	59	(32.1)		NS	NS
TAP1*C	3	(5.7)	18	(9.8)		NS	NS
Allele frequencies							
	(2 <i>n</i> = 106)		(2 <i>n</i> = 368)				
TAP1*A	84	(79.2)	286	(77.7)		NS	NS
TAP1*B	19	(17.9)	63	(17.1)		NS	NS
TAP1*C	3	(2.8)	19	(5.2)		NS	NS

NS, statistically not significant.

polymorphisms was similar in atopic dermatitis patients and controls.

TAP2 genotype, phenotype, and allele frequencies are described in Table 3. TAP2*C allele and TAP2*A/TAP2*C genotype were found to be significantly increased in patients with atopic dermatitis ($P < 0.05$, $P_c < 0.05$).

LMP2 and LMP7 polymorphisms in atopic dermatitis patients

Genotype, phenotype and allele frequencies of LMP2 and LMP7 genes were not significantly different for atopic dermatitis patients and controls (Tables 4 and 5).

Discussion

Studies have focused on genetic variation with the HLA genes and atopic disorders because HLA molecules are involved in the recognition and presentation of allergen in immune response. Earlier studies identified an increased frequency of specific combinations of HLA types with either total serum IgE levels or an atopic disorder [29].

In Japan, atopic dermatitis patients were found to have increased frequencies of HLA-A24, A33, and B44, but without statistical significance [18,19]. In this study, atopic dermatitis patients showed significantly higher frequencies of HLA-A24 than controls.

Comparisons between studies are complicated by differences in the ethnic group studied, the phenotypic definitions, and the HLA typing methods [30]. In this study,

HLA frequencies were investigated in heterogenous atopic dermatitis patients with various serum IgE levels, family or personal history of atopic disorders including bronchial asthma and allergic rhinitis, and skin prick test to common allergens. These characteristics are believed to have significant influences in interpreting the HLA or other genetic associations. Analysis of HLA allele frequencies in subgroups with different clinical and laboratory characteristics might reveal characteristic distributions, and these results could be used as additional criteria for defining atopic dermatitis subgroups and determining more individualized therapeutic modalities.

TAP gene polymorphisms have been found in various diseases including atopic dermatitis. TAP gene polymorphisms were reported to alter the peptide processing pathway, however, TAP gene association has not been found in ankylosing spondylitis [8], celiac disease [8,9], and insulin-dependent diabetes mellitus [8,31]. The frequency of TAP 1637 Asp of TAP1*A or TAP1*C showed a tendency to increase in the patients with atopic dermatitis (95%) in Japanese population compared with control subjects (79%) [4], although the result was not statistically significant after *P*-values were corrected by multiplying by the number of epitopes tested. A subsequent study by the same investigators found no differences in TAP1 and TAP2 allele frequencies between atopic dermatitis and Japanese control subjects [11]. Discrepancy in TAP gene association between our and Kuwata *et al.*'s results might be related to different patient characteristics. In Japanese studies, severe atopic dermatitis patients were included with high

Table 3. Distribution of TAP2 alleles in atopic dermatitis patients

	Patients (<i>n</i> = 53)		Controls (<i>n</i> = 184)		RR (95% CI)	<i>P</i> -value	<i>P</i> _c -value
	<i>n</i>	(%)	<i>n</i>	(%)			
Genotype frequencies							
TAP2*A/TAP2*A	10	(18.9)	35	(19.0)		NS	NS
TAP2*A/TAP2*B	13	(24.5)	66	(35.9)		NS	NS
TAP2*A/TAP2*C	10	(18.9)	8	(4.3)	5.1163 (2.01–12.45)	0.002	0.028
TAP2*A/TAP2*E	3	(5.7)	9	(4.9)		NS	NS
TAP2*B/TAP2*B	6	(11.3)	17	(9.2)		NS	NS
TAP2*B/TAP2*E	3	(5.7)	12	(6.5)		NS	NS
TAP2*B/TAP2*G	0	(0.0)	3	(1.6)		NS	NS
TAP2*C/TAP2*C	1	(1.9)	1	(0.5)		NS	NS
TAP2*C/TAP2*D	0	(0.0)	2	(1.1)		NS	NS
TAP2*D/TAP2*E	0	(0.0)	1	(0.5)		NS	NS
TAP2*E/TAP2*E	1	(1.9)	1	(0.5)		NS	NS
TAP2*A/TAP2*G or TAP2*B/TAP2*C ¹	5	(9.4)	22	(12.0)		NS	NS
TAP2*B/TAP2*D or TAP2*E/TAP2*G ¹	1	(1.9)	4	(2.2)		NS	NS
TAP2*A/TAP2*D or TAP2*C/TAP2*E ¹	0	(0.0)	3	(1.6)		NS	NS
Phenotype frequencies							
TAP2*A	36	(67.9)	118	(64.1)		NS	NS
TAP2*B	22	(41.5)	98	(53.3)		NS	NS
TAP2*C	11	(20.8)	11	(6.0)	4.1190 (1.76–9.63)	0.003	0.027
TAP2*D	0	(0.0)	3	(1.6)		NS	NS
TAP2*E	7	(13.2)	23	(12.5)		NS	NS
TAP2*G	0	(0.0)	3	(1.6)		NS	NS
Allele frequencies							
	(2 <i>n</i> = 106)		(2 <i>n</i> = 368)				
TAP2*A	46	(43.4)	153	(41.6)		NS	NS
TAP2*B	28	(26.4)	115	(31.3)		NS	NS
TAP2*C	12	(11.3)	12	(3.3)	3.7872 (1.73–8.28)	0.003	0.027
TAP2*D	0	(0.0)	3	(0.8)		NS	NS
TAP2*E	8	(7.5)	24	(6.5)		NS	NS
TAP2*G	0	(0.0)	3	(0.8)		NS	NS

NS, statistically not significant.¹heterozygotes cannot be distinguished by the typing method used in this study.

serum IgE levels in the ranges of 10 500–75 600 U/mL (mean $\pm$ SD, 26 500 $\pm$ 34 200 U/mL) [4] or 8 000–75 600 U/mL (mean $\pm$ SD, 25 400 $\pm$ 28 200 U/mL) [11]. But, in this study, our Korean atopic dermatitis patients were not selected according to serum IgE levels and consisted of subjects with IgE levels ranging from 22 to 2500 IU/mL (mean $\pm$ SD 646.1 $\pm$ 581.4 IU/mL). Serum IgE levels in our patients were much lower than those in Japanese patients and this difference in serum IgE level

might be a cause of different TAP1 gene association, although Ismail *et al.* [23] could not find association of TAP1 polymorphism with regard to total serum IgE. TAP gene involvement in the control of serum IgE production should be addressed in a further study. In a recent study in Tunisian population [23], TAP1 Val-333 and Gly-637 codons were reported to be associated with an increased risk of atopy, resulting in an increased frequency of TAP1*B allele in atopic dermatitis patients. TAP1*B/TAP1*B and

Table 4. Distribution of LMP2 alleles in atopic dermatitis patients

	Patients (<i>n</i> = 53)		Controls (<i>n</i> = 184)		RR (95% CI)	<i>P</i> -value	<i>P</i> _c -value
	<i>n</i>	(%)	<i>n</i>	(%)			
Genotype frequencies							
LMP2*R/LMP2*R	32	(60.4)	113	(61.4)		NS	NS
LMP2*R/LMP2*H	20	(37.7)	64	(34.8)		NS	NS
LMP2*H/LMP2*H	1	(1.9)	7	(3.8)		NS	NS
Phenotype frequencies							
LMP2*R	52	(98.1)	177	(96.2)		NS	NS
LMP2*H	21	(39.6)	71	(38.6)		NS	NS
Gene frequencies	(2 <i>n</i> = 106)		(2 <i>n</i> = 368)				
LMP2*R	84	(79.2)	290	(78.8)		NS	NS
LMP2*H	22	(20.8)	78	(21.2)		NS	NS

NS, statistically not significant.

TAP1*A/TAP1*D genotypes showed significantly higher frequencies in atopic dermatitis patients than controls (10% vs. 0% for the TAP1*B/TAP1*B genotype and 20% vs. 6% for the TAP1*A/TAP1*D genotype), but TAP1*D allele was not associated with atopic dermatitis. No association of TAP1 polymorphism was found in atopic patients with

regard to total serum IgE and it was suggested that the TAP1 gene was not involved in the control of total IgE production [23]. At this point, we cannot exclude the possibility that TAP2 gene association could be secondary due to linkage disequilibrium between TAP and HLA class II genes.

Table 5. Distribution of LMP7 alleles in atopic dermatitis patients

	Patients (<i>n</i> = 53)		Controls (<i>n</i> = 184)		RR (95% CI)	<i>P</i> -value	<i>P</i> _c -value
	<i>n</i>	(%)	<i>n</i>	(%)			
Genotype frequencies							
LMP7*A/LMP7*A	9	(17.0)	26	(14.1)		NS	NS
LMP7*A/LMP7*B	27	(50.9)	78	(42.4)		NS	NS
LMP7*B/LMP7*B	14	(26.4)	56	(30.4)		NS	NS
LMP7*B/LMP7*C	2	(3.8)	16	(8.7)		NS	NS
LMP7*C/LMP7*C	0	(0.0)	1	(0.5)		NS	NS
LMP7*D/LMP7*X ¹	1	(1.9)	7	(3.8)		NS	NS
Phenotype frequencies							
LMP7*A	36	(67.9)	104	(56.5)		NS	NS
LMP7*B	43	(81.1)	150	(81.5)		NS	NS
LMP7*C	2	(3.8)	17	(9.2)		NS	NS
LMP7*D	1	(1.9)	7	(3.8)		NS	NS
Allele frequencies	(2 <i>n</i> = 106)		(2 <i>n</i> = 368)				
LMP7*A	45	(42.5)	130	(35.3)		NS	NS
LMP7*B	57	(53.8)	206	(56.0)		NS	NS
LMP7*C	2	(1.9)	16	(4.3)		NS	NS
LMP7*D	0	(0.0)	0	(0.0)		NS	NS

NS, statistically not significant.

¹LMP7*X is an LMP7*A, B, C or D gene that cannot be designated as a single allele by the PCR-RFLP method adopted here when combined with LMP7*D. Subjects with LMP7*X were therefore excluded from calculating LMP7 allele frequencies in the present study.

Interestingly, this study showed increased frequencies of TAP2*C allele and TAP2*A/TAP2*C genotype in patients with atopic dermatitis compared to controls. TAP2*A allele distribution was not statistically different between patients and controls. TAP2 gene association has not been reported in atopic dermatitis. Allele frequency of TAP2*C in Japanese patients [11] with atopic dermatitis (15%) showed no differences compared with controls (10%). Although this result suggests a role of TAP2 alleles in atopic dermatitis in Koreans, their functional or pathogenetic significance is still uncertain.

TAP1 allele distributions in Korean patients and controls were similar to those in Japanese, Tunisian and Caucasian [23] populations, and ethnic differences were not prominent. TAP2 alleles also showed similar distributions in Korean, Japanese and Caucasian populations with the exception of TAP2*E allele, which was higher in Korean (6.5%, $P < 0.00004$) and Japanese (11%) [11] control subjects than in Caucasians (1%) [32].

LMP genes are closely linked to MHC class II genes and are believed to be involved in the antigen processing pathway. Polymorphisms in the amino acid composition of the LMP2 and LMP7 proteins have been reported in humans [33–35]. Controversial results were reported on the association of the LMP2 gene polymorphism with extraspinal disease in HLA-B27 positive subjects with ankylosing spondylitis [10,36–38]. LMP genes were rarely studied in patients with atopic dermatitis although these molecules are involved in the antigen processing pathway. In our study, LMP2 and LMP7 alleles showed similar distributions in atopic dermatitis patients and controls.

In summary, HLA-A24 was significantly increased in atopic dermatitis patients. TAP2*C allele and TAP2*A/TAP2*C genotype showed higher frequencies in atopic dermatitis patients. TAP1 alleles, and LMP2 and LMP7 alleles were similar in atopic dermatitis patients and controls. These results differ from those of previous reports and the discrepancy might be due to different phenotypic characteristics, heterogeneity within the group of patients, and ethnic variations. However, further studies are needed in larger numbers of patients to determine the functional implications of these genes.

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