

SUPPLEMENT TO
THE JOURNAL OF
ALLERGY
AND
CLINICAL IMMUNOLOGY

VOLUME 96

NUMBER 6, PART 2

Session I: Allergy and Genes

Lack of primary association between transporter associated with antigen processing genes and atopic dermatitis

Shoji Kuwata, MD,^a Masami Yanagisawa, BS,^a Hidehisa Saeki, MD,^b
Hidemi Nakagawa, MD,^b Takafumi Etoh, MD,^b Katsushi Tokunaga, PhD,^c
Takeo Juji, MD,^c and Yoichi Shibata, MD^a Tokyo, Japan

We examined polymorphisms of transporter associated with antigen processing (TAP) genes in 37 Japanese patients with atopic dermatitis and 52 control subjects. We have evaluated, again, the specificity polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method since our previous report and have also developed a mismatch PCR-RFLP method for discriminating two dimorphic sites of TAP1 gene and four dimorphic sites of TAP2 gene. Amplified products from genomic DNA were digested with restriction endonucleases: Sau3AI for TAP1 codon 333 (Ile-Val), AccI for TAP1 codon 637 (Asp-Gly), and BfaI for TAP2 codon 687 (Stop-Gln). The other sites of TAP2 gene were analyzed by mismatch PCR-RFLP: AccII for TAP2 codon 379 (Val-Ile), RsaI for codon 565 (Ala-Thr), and MspI for codon 665 (Thr-Ala). We observed three TAP1 alleles and six TAP2 alleles. Infrequent allele TAP1 C was observed in one patient and one control subject. We did not identify any differences in TAP allele frequencies between those patients with atopic dermatitis and Japanese control subjects. Analysis of TAP gene polymorphisms will provide better understanding of susceptibility loci in HLA class II-associated disease because TAP genes are located between HLA-DQB1 and HLA-DPB1 loci. (J ALLERGY CLIN IMMUNOL 1995;96:1051-60.)

Key words: TAP, HLA, PCR, PCR-RFLP, mismatch PCR-RFLP, atopic dermatitis, haplotype, linkage disequilibrium, Japanese

From ^aDepartment of Transfusion Medicine and Immunohematology, Faculty of Medicine, University of Tokyo; ^bDepartment of Dermatology, Faculty of Medicine, University of Tokyo; and ^cJapanese Red Cross Central Blood Center, Tokyo.

Supported in part by a research grant from New Trends In Immunopharmacology and a research grant for Intractable Disease from Ministry of Health and Welfare, Japan.

Reprint requests: Shoji Kuwata, MD, Department of Transfusion Medicine and Immunohematology, Faculty of Medicine, University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113, Japan.

Copyright © 1995 by Mosby–Year Book, Inc.

0091-6749/95 \$5.00 + 0 1/0/68348

Antigenic peptides are presented to self-restricted cytotoxic T lymphocytes by the HLA class I molecules. These peptides are degraded from cytosolic proteins and assembled with HLA class I heavy chain and β_2 -microglobulin in the endoplasmic reticulum (ER). The peptides are actively transported across the ER membrane by the transporter associated with antigen processing (TAP).¹⁻⁴ TAP genes consist of TAP1 and TAP2 genes and encode a heterodimer molecule, which is responsible for translocation peptides.

Abbreviations used

AD:	Atopic dermatitis
ER:	Endoplasmic reticulum
MHC:	Major histocompatibility complex
PCR:	Polymerase chain reaction
PCR-RFLP:	Polymerase chain reaction–restriction fragment length polymorphism
TAP:	Transporter associated with antigen processing

TAP gene polymorphisms have been reported since the discovery of genes.^{5,6} Each of the two dimorphic sites in the TAP1 gene encodes one of two possible amino acids. One of the four dimorphic sites in the TAP2 gene encodes a transition that creates a premature stop codon. Each of the other three remaining sites also encodes two possible amino acids. Diverse combinations of polymorphisms at these sites constitute four possible alleles at TAP1 and eight possible alleles at TAP2.⁷ TAP gene polymorphisms are supposed to cause alterations in the peptide processing pathway by selecting the peptide by its size and amino acid sequence.⁸

The TAP gene is considered to be one of the candidates for disease susceptibility gene or one of the additional genes conferring susceptibility. Polymorphisms of the TAP gene have been investigated in several HLA-associated diseases. We have reported our preliminary data on the association of TAP alleles with atopic dermatitis (AD).⁹ After our preliminary report, we further evaluated the specificity of the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method and also developed a mismatch PCR-RFLP method to create the nucleotide sequence recognized by restriction endonucleases in PCR products. We were able to identify only one dimorphic site of the TAP2 gene, using the conventional PCR-RFLP method, in our previous report. In this study we were able to examine the other three dimorphic sites of the TAP2 gene by introducing the sequences recognized by restriction endonucleases. Analysis with PCR-RFLP and mismatch PCR-RFLP will facilitate the determination of TAP alleles exactly, quickly, and easily.

Patients with AD generated IgE against several environmental allergens.¹⁰ CD4⁺ helper T cells play a role in B cell-mediated immunity in the

context of the HLA class II–restricted antigen recognition pathway. On the other hand, CD8⁺ cytotoxic T lymphocytes predominantly infiltrate the skin lesions of active AD in the context of HLA class I–restricted antigen recognition. Both HLA class I and class II antigens are supposed to contribute to the pathogenesis of AD. Analysis of the TAP gene will contribute to illuminating the antigenic peptide processing pathway in AD. Japanese patients with AD showed a tendency to an increase in HLA-DR13/DQ6.¹¹ Because TAP genes are located in the HLA class II region between DPB1 and DQB1 loci, we further estimated extended HLA haplotypes, including TAP genes, in the Japanese population.

METHODS

Patients

We studied 37 Japanese patients with AD who were admitted to the Department of Dermatology at the University of Tokyo Hospital. The study group consisted of 27 male and 10 female patients, aged 11 to 42 years (mean age, 27.0 ± 6.2 years). All patients manifested a severe clinical form of AD and demonstrated high serum IgE levels in the range of 8000 to 75,600 U/ml (mean level, $25,400 \pm 28,200$ U/ml). Fifty-two Japanese subjects without atopic constitutions served as control subjects. Venous blood was drawn from each individual after informed consent was obtained.

HLA typing

Serologic HLA typing was performed in all patients and control subjects by means of the standard lymphocyte microcytotoxicity test. HLA-DRB1 and -DQB1 alleles were also determined by the PCR–sequence-specific oligonucleotide (RFLP) method with genomic DNA. Primers and restriction endonucleases are described elsewhere.¹²

PCR for TAP genes

Oligonucleotide primers for PCR were synthesized with the methoxyphosphoramidite method on an Applied Biosystems 381A DNA Synthesizer (Applied Biosystems, Foster City, Calif.). We used two sets of primers for the TAP1 gene and three sets of primers for the TAP2 gene. Nucleotide sequences of the primers are shown in Table I. We amplified two regions of the TAP1 gene and three regions of the TAP2 gene by PCR and tentatively designated these regions as TAP1-1, TAP1-2, TAP2-1, TAP2-2, and TAP2-3. The TAP1-1 region contains a 235 bp fragment spanning exon 4 and intron 4 of the TAP1 gene, including dimorphic site at codon 333 (Ile-Val). The TAP1-2 region contains a 183 bp fragment spanning intron 9 and exon 10 of the TAP1 gene, including a dimorphic site at codon 333 (Asp-Gly).

TABLE I. A panel of primers for amplification of TAP genes

Primers	Nucleotide sequences	Dimorphic sites
<i>TAP1 gene</i>		
TAP1-1AMPA:	5'-CACCCTGAGTGATTCTCT-3'	TAP1 codon 333
TAP1-1AMPB:	5'-ACTGAGTCTGCCAAGTCT-3'	
TAP1-2AMPA:	5'-CCCTATCCAGCTACAACC-3'	codon 637
TAP1-2AMPB:	5'-AACGCCACTGCCTGTCGCT-3'	
<i>TAP2 gene</i>		
TAP2-1MAMPA*:	5'-GAACGTCCTTGTACCTGCGC-3'	TAP2 codon 565
TAP2-1AMPB:	5'-ACCCCAAGTGCAGCAC-3'	
TAP2-2MAMPA*:	5'-GGTGATTGCTCACAGGCTGCCG-3'	codons 665 and 687
TAP2-2AMPB:	5'-CACAGCTCTAGGGAACTC-3'	
TAP2-3MAMPA*:	5'-CCGGTTCTGTGAGGAACAACAGT-3'	codon 379
TAP2-3AMPB:	5'-GGAGCAAGCTTACAATTTGT-3'	

*Discordant nucleotide sequences in each mismatched primer are indicated as underlined.

The TAP2-1 region contains a 212 bp fragment spanning exon 5, intron 5, and exon 6 of the TAP2 gene, including a dimorphic site at codon 379 (Val-Ile). The TAP2-2 region contains a 227 bp fragment spanning exon 11 and intron 11 of the TAP2 gene, including dimorphic sites at codon 665 (Thr-Ala) and codon 687 (Stop-Gln). The TAP2-3 region contains a 155 bp fragment spanning exon 9 and intron 9 of the TAP2 gene, including dimorphic site at codon 565 (Ala-Thr). A forward primer for each TAP2 region contains mismatch oligonucleotide sequences to create the sequences recognized by restriction endonucleases.

PCR was carried out as described by Saiki et al.¹³ with minor modifications. Genomic DNA (0.2 µg) was suspended in 20 µl of 50 mmol/L Tris/HCl (pH 8.3), 1.5 mmol/L MgCl₂, 200 nmol/L each deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP), 10 pmol each primer, and 0.4 U Takara-Taq DNA polymerase (Takara-shuzo, Tokyo, Japan). PCR cycles consisted of an initial 2-minute denaturation at 95° C and 30 cycles of 1-minute denaturation at 95° C, 1-minute annealing at 52° C, and 1-minute extension at 72° C, followed by 5-minute extension at 72° C. This cycling was performed in a programmable DNA Thermal Cycler (Perkin Elmer Cetus, Norwalk, Conn.).

Genotyping of dimorphisms of TAP1 and TAP2 by PCR-RFLP

Amplified PCR products were subjected to digestion with restriction endonucleases. Five milliliters of PCR-amplified products was digested with each enzyme in a total volume of 10 µl in 5 to 10 U of restriction endonuclease and the reaction buffer provided by the manufacturers. Dimorphisms at TAP1 codon 333 (TAP1-1) and at codon 637 (TAP1-2) were discerned by digestion with *Sau3AI* and *AccI*, respectively. Dimorphism at TAP2 codon 687 (TAP2-2) was detected by digestion with *BfaI*, which replaced *EcoRI* as previously reported. The other three dimorphisms of TAP2 genes were detected by mismatch PCR-RFLP with *AccII*

(codon 379; TAP2-1), *MspI* (codon 665; TAP2-2), and *RsaI* (codon 565; TAP2-3). After 2 hours of digestion reaction, fragments were fractionated in 10% polyacrylamide gel in 1× Tris-borate-EDTA buffer at 200 V and visualized by staining with ethidium bromide.

Four possible TAP1 alleles were determined by a combination of two dimorphic sites at codon 333 (Ile-Val) and codon 637 (Asp-Gly). Eight possible TAP2 alleles were determined by a combination of four dimorphic sites at codon 379 (Val-Ile), codon 565 (Ala-Thr), codon 665 (Thr-Ala), and codon 687 (Stop-Gln). TAP1 and TAP2 allele nomenclature, as proposed by Powis et al.,^{6,7} is shown in Fig. 1.

We examined the dimorphic sites by direct sequencing and confirmed the obtained sequences by comparison with those reported by Beck et al.¹⁴ Fractionated PCR fragments from homozygous samples at each dimorphic site were cut in 1.5% Nusieve GTG agarose gel (FMC Bio-Products, Rockland, Maine) and purified. Direct sequencing was performed by using the Ampli-Taq-facilitated cycle sequencing reaction with the dideoxy terminator method (Applied Biosystems) according to the manufacturer's recommendations. Nucleotide sequences were obtained with an Applied Biosystems 373A automated DNA sequencer. Both strands were sequenced with the same primers as used in PCR amplification.

Estimation of haplotype including TAP alleles

Because TAP gene loci are located in the HLA class II region, we estimated haplotypes, including HLA-DRB1*1302/DQB1*0604 and TAP alleles, in the patients and the control subjects. Statistical significance was calculated with the chi square test according to the report by Djilali et al.¹⁵ The delta value (Δ) of the linkage disequilibrium was assessed with the formula:

$$\Delta AB =$$

$$\sqrt{A^-B^-} - \sqrt{(A^-B^+ + A^-B^-)(A^+B^- + A^-B^-)}$$

		codon			
		333	637		
TAP1	A	Ile	Asp		
	B	Val	Gly		
	C	Val	Asp		
	D	Ile	Gly		
		codon			
		379	565	665	687
TAP2	A	Val	Ala	Thr	Stop
	B	Val	Ala	Ala	Gln
	C	Ile	Ala	Thr	Stop
	D	Ile	Thr	Thr	Stop
	E	Val	Thr	Thr	Stop
	F	Ile	Thr	Ala	Gln
	G	Val	Thr	Ala	Gln
	H	Ile	Ala	Ala	Gln

FIG. 1. Nomenclature for TAP alleles. TAP nomenclature is described according to the reports of Powis et al.^{6,7} Combinations of each dimorphic site constitute four TAP1 alleles and eight TAP2 alleles.

where A and B are the observed frequency of each allele.¹⁵

Nomenclature

Nomenclature for HLA specificities and HLA class II alleles was described according to the guidelines of the World Health Organization Nomenclature Committee, HLA 1994.¹⁶ Nomenclature for TAP alleles was described according to the reports of Powis et al.^{6,7}

Statistics

Frequencies of each allele or amino acid residue were compared by using the chi square test. The odds ratio of the disease was calculated according to the method of Svejgaard et al.¹⁷ and expressed as relative risk. Statistical calculations were performed with a Macintosh IICI

personal computer (Apple Computer, Inc. Cupertino, Calif.) and StatView software version 4.0 (Abacus Concepts, Inc., Berkeley, Calif.) with the System.7.1 version.

RESULTS

Resolution of dimorphic site of TAP genes

We determined the expected size of digested fragments by using the PCR-RFLP method based on the nucleotide sequences of TAP genes. *Sau3AI* digestion of TAP1-1 region (codon 333) produced fragments of 146 bp, 74 bp, and 15 bp for Val (GTC) or fragments of 118 bp, 74 bp, 28 bp, and 15 bp for Ile (ATC). *AccI* digestion of TAP1-2 region (codon 637) produced fragments of 132 bp and 51 bp for Asp (GAC) or a 183 bp undigested fragment for Gly (GGC) (Fig. 1). *BfaI* digestion of TAP2-2 region (codon 687) produced fragments of 127 bp, 88 bp, and 12 bp for Stop codon (TAG) or fragments of 215 bp and 12 bp for Gln (CAG). The other three regions were analyzed with the mismatch PCR-RFLP method. *AccII* digestion of TAP2-1 region (codon 379) produced fragments of 192 bp and 20 bp for Val (GCT) or a 212 bp undigested fragment for Ile (ATA). *MspI* digestion of TAP2-2 region (codon 665) produced fragments of 207 bp and 20 bp for Thr (ACA) or a 227 bp undigested fragment for Ala (GCA). *RsaI* digestion of TAP2-3 region (codon 565) produced fragments of 132 bp and 23 bp for Thr (ACT) or a 155 bp fragment for Ala (GCT) (Fig. 2). PCR-RFLP and mismatch PCR-RFLP analysis were resolved to work completely and to produce fragments the same size as those expected in homozygous sequenced samples. Representative results of PCR-RFLP are shown in Figs. 2 and 3.

Amino acid residue frequencies of TAP molecules

We examined amino acid residue frequencies at each dimorphic site of TAP molecules, and the results are shown in Table II. Phenotype or gene frequency of any of the amino acids did not show a tendency to increase in the patients with AD.

Genotype and allele frequencies of TAP genes

We also estimated genotype and allele frequencies of the TAP gene. Our estimation is shown in Tables III, IV, and V. When heterozygosities for both codon 333 Val/Gly and codon 637 Ile/Asp of TAP1 were observed in a single individual, two possible allele combinations of TAP1 A/TAP1 B or

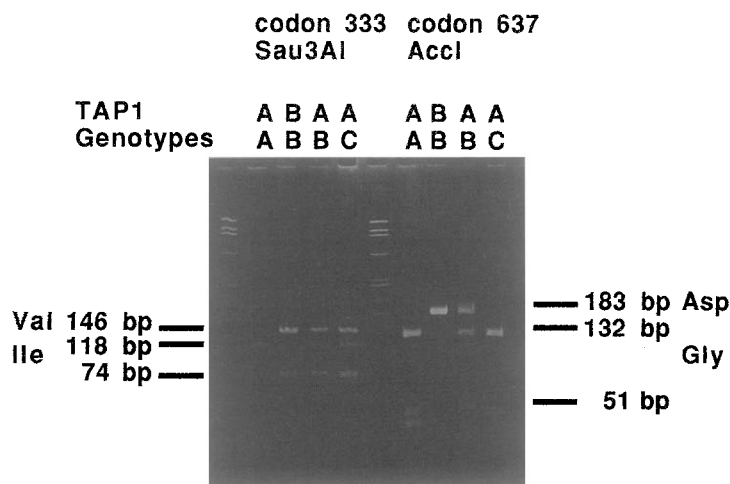


FIG. 2. PCR-RFLP analysis of TAP1 gene dimorphisms. PCR products digested with restriction endonucleases *Sau3AI* and *AccI* were electrophoresed in 10% polyacrylamide gel. This figure represents *Sau3AI*-digested fragment patterns on the left and *AccI*-digested fragment patterns on the right. Lanes 1 and 6 contain DNA size marker, *HaeIII*-digested ϕ x174. Lanes 2 and 7 contain samples with TAP1 AA genotype; lane 3 and 8, samples with TAP1 BB; lanes 4 and 9, samples with TAP1 AB; and lanes 5 and 10, samples with TAP1 AC.

TAP1 C/TAP1 D were considered. However, a combination of TAP1 C/TAP1 D was estimated to be very rare. We regarded this as a combination of TAP1 A/TAP1 B as proposed by Powis et al.^{6,7} TAP1 C allele was observed in one of 74 total alleles in the patients with AD and also in one of a total of 104 alleles in the control subjects. As for TAP2 allele estimation, it was done in the heterozygotes at three or four dimorphic sites. When heterozygosities for codon 379 Ile/Val, codon 665 Thr/Ala, and codon 687 Gln/Stop were observed in a single individual, two possible allele combinations of TAP2 B/TAP2 C or TAP2 A/TAP2 H were considered. However, a combination of TAP2 A/TAP2 H was estimated to be very rare, and we regarded this as a combination of TAP2 B/TAP2 C. When heterozygosities for codon 565 Ala/Thr, codon 665 Thr/Ala, and codon 687 Gln/Stop were observed in a single individual, we regarded this as a combination of TAP2 B/TAP2 E among two possible allele combinations of TAP2 B/TAP2 E or TAP2 A/TAP2 G. In addition, heterozygosities for both codon 379 Ile/Val and codon 565 Ala/Thr with codon 665 Thr and codon 687 Stop were observed in two patients with AD. Two possible allele combinations of TAP2 A/TAP2 D or TAP2 C/TAP2 E were considered in this patient. However, a combination of TAP2 C/TAP2 E was estimated to be very rare, and we regarded this as a combination of TAP2 A/TAP2 D.

Estimation of haplotypes between HLA and TAP alleles

We examined whether TAP alleles were in linkage disequilibrium with DRB1/DQB1 alleles. The results are summarized in Table VI. We scrutinized the association between DRB1*1302/DQB1*0604 and TAP alleles. We were unable to find any possible linkage disequilibrium between the two loci.

DISCUSSION

We defined the TAP1 and TAP2 alleles in patients with AD in this study. Because the Japanese patients with AD had a tendency toward an increase in the frequency of HLA-DR13/DQ6,¹¹ a possible association with TAP alleles was investigated. We were unable to find any association of the frequencies of TAP allele and amino acid residue. In one of 37 patients and one of 52 control subjects, we observed TAP1 C allele, which has also been reported as an infrequent allele in Caucasian populations.^{6,7} At the codon 687 dimorphic site of the TAP2 gene, CAG(Gln) or TAG(Stop) transition was observed. TAP2 allele with stop codon at amino acid position 687 expresses a shorter molecule in 17 amino acids than an allele with 687 Gln. Again, we did not find differences in frequencies of these epitopes between patients and control subjects. Furthermore, frequencies of TAP alleles or TAP epitopes were estimated to be

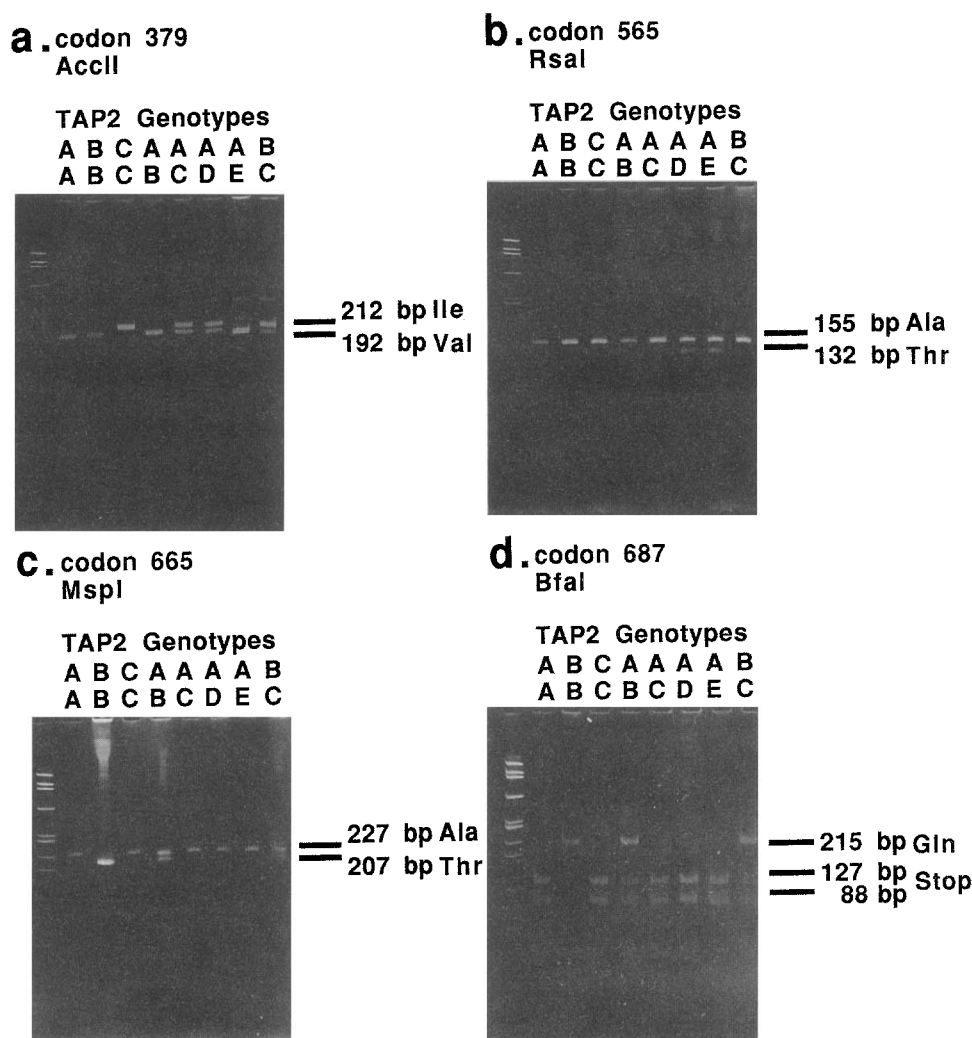


FIG. 3. PCR-RFLP and mismatch PCR-RFLP analysis of TAP2 gene dimorphisms. PCR products digested with restriction endonucleases *AccII* (a), *RsaI* (b), *MspI* (c), and *BfaI* (d) were electrophoresed in 10% polyacrylamide gel. Each lane 1 contains DNA size marker, *HaeIII*-digested ϕ x174. In each gel lanes 2 to 9 correspond to TAP2 genotypes, AA, BB, CC, AB, AC, AD, AE, and BC, respectively.

similar between the Japanese and Caucasians populations, whereas diverse ethnic differences have been known in frequencies of HLA alleles.

Because TAP genes are located in the HLA class II region between DPB1 and DQB1 loci, linkage disequilibrium between HLA class II alleles and TAP alleles is expected. We also estimated the extended HLA haplotypes, including TAP genes, in both the patients and control subjects. Any TAP alleles in combination with DR13-DQ6 did not reach significance in our study. In Caucasian populations haplotypes between HLA-DR/DQ and TAP alleles were reported^{5-7, 18}: DRB1*0101-DQB1*0501-TAP2B, DRB1*1501-DQB1*0602-TAP1A, DR3-DQ2-TAP1A-TAP2A, DRB1*

0401-DQB1*0302-TAP2A, DRB1*1101-DQB1*0301-TAP1A, DR7-DQ2-TAP1A-TAP2A, and DRB1*0801-DQB1*0402-TAP2C. Because DR3 and DR7 haplotypes are rare in Japanese, more extensive study is needed to define the HLA-TAP haplotypes in the Japanese population.

In this study we used the PCR-RFLP method with restriction endonucleases to discriminate TAP alleles. TAP1 allele frequencies in our previous preliminary reports⁹ were considerably different from those in this report. This may derive from the partial digestion of restriction endonucleases. *Sau3A1* and *AccI* require special buffers for optimal DNA digestion. In the former, partial digestion by conventional reaction buffer may occur and

TABLE II. TAP amino acid residue frequencies in AD

1. Phenotype frequencies

Amino acid		Patients with AD (n = 37)	Control subjects (n = 52)	Relative risk	Chi square	p Value
TAP1						
codon 333	Val	10 (27%)	14 (27%)	1.01	0.0001	0.9913
	Ile	36 (97%)	50 (96%)	1.44	0.0000	1.0000
codon 637	Gly	9 (24%)	13 (25%)	0.96	0.0053	0.9419
	Asp	36 (97%)	50 (96%)	1.44	0.0000	1.0000
TAP2						
codon 379	Ile	13 (35%)	11 (21%)	2.02	2.1457	0.1430
	Val	36 (97%)	52 (100%)	0.00	0.0296	0.8635
codon 565	Ala	37 (100%)	50 (96%)	3.71	0.2314	0.6305
	Thr	5 (14%)	11 (21%)	0.58	0.8558	0.3549
codon 665	Thr	35 (95%)	47 (90%)	1.86	0.1074	0.7432
	Ala	15 (41%)	31 (60%)	0.46	3.1499	0.0759
codon 687	Gln	15 (41%)	31 (60%)	0.46	3.1499	0.0759
	Stp	35 (95%)	47 (90%)	1.86	0.1074	0.7432

2. Gene frequencies

Amino acid		Patients with AD (n = 74)	Control subjects (n = 104)	Relative risk	Chi square	p Value
TAP1						
codon 333	Val	11 (15%)	16 (15%)	0.96	0.0091	0.9241
	Ile	63 (85%)	88 (85%)	1.04	0.0091	0.9241
codon 637	Gly	10 (14%)	15 (14%)	0.93	0.0296	0.8633
	Asp	64 (87%)	89 (86%)	1.08	0.0296	0.8633
TAP2						
codon 379	Ile	14 (19%)	11 (11%)	1.97	2.4923	0.1144
	Val	60 (81%)	93 (89%)	0.51	2.4923	0.1144
codon 565	Ala	68 (92%)	91 (88%)	1.62	0.8747	0.3497
	Thr	6 (8%)	13 (13%)	0.62	0.8747	0.3497
codon 665	Thr	57 (77%)	68 (65%)	1.78	2.8028	0.0941
	Ala	17 (23%)	36 (35%)	0.56	2.8028	0.0941
codon 687	Gln	17 (23%)	36 (35%)	0.56	2.8028	0.0941
	Stp	57 (77%)	68 (65%)	1.78	2.8028	0.0941

cause overestimation and underestimation of TAP1 allele frequencies. We also replaced the restriction enzyme *EcoRII* with *BfaI* for detecting TAP2 codon 687 transitions. Because *EcoRII* produced smaller fragments, it was not always easy to discriminate between fragments of 60 bp and 53 bp. In addition, we have developed the mismatch PCR-RFLP method to create the nucleotide sequences recognized by restriction endonucleases. The conventional PCR-RFLP method did not work in the nucleotide region that did not contain recognition sequences. Bijvoet and Hayden¹⁹ first introduced the mismatch PCR-RFLP method to detect codon 207 Pro or Leu mutation in the lipoprotein lipase gene. The mismatch PCR-RFLP method will provide further applications for analyzing other polymorphic genes or gene mutations.

PCR-RFLP and mismatch PCR-RFLP will provide an alternative method, replacing sequence-specific or allele amplification designated as an amplification refractory mutation system,⁶ sequence-specific oligonucleotide,⁵ and single-strand conformation polymorphisms.⁵ Furthermore, PCR amplification, encompassing relatively large fragments, is also being examined to determine TAP alleles exactly, by defining simultaneously different dimorphic sites of TAP genes.

TAP gene polymorphisms have, to date, been investigated in several HLA class II-associated diseases in Caucasian patients. Investigations of celiac disease,^{7, 20} multiple sclerosis,^{21, 22} and insulin-dependent diabetes mellitus^{18, 23} did not demonstrate significant association with TAP alleles. No report, so far, has demonstrated a significant

TABLE III. TAP genotype frequencies in AD

TAP genotypes	Patients with AD (n = 37)	Control subjects (n = 52)	Relative risk	Chi square	p Value
TAP1 AA	27 (73%)	38 (73%)	0.99	0.0001	0.9913
BB	1 (3%)	2 (4%)	0.69	0.0000	1.0000
AB	8 (22%)	11 (21%)	1.03	0.0028	0.9577
AC	1 (3%)	1 (2%)	1.42	0.0000	1.0000
TAP2 AA	10 (27%)	11 (21%)	1.38	0.4136	0.5201
AB	11 (30%)	16 (31%)	0.95	0.0111	0.9163
AC	7 (19%)	4 (8%)	2.80	2.5154	0.1127
AD	2 (8%)	0 (0%)	30.25	9.8521	0.0017
AE	2 (5%)	4 (8%)	0.69	0.0000	1.0000
BB	2 (5%)	4 (8%)	0.69	0.0000	1.0000
BC	2 (5%)	6 (12%)	0.44	0.3856	0.5346
CC	1 (3%)	0 (0%)	4.32	0.0296	0.8635
BE	0 (0%)	4 (8%)	0.00	1.4574	0.2273
BG	0 (0%)	1 (2%)	0.00	0.0000	1.0000
DE	0 (0%)	1 (2%)	0.00	0.0000	1.0000
EE	0 (0%)	1 (2%)	0.00	0.0000	1.0000

TABLE IV. TAP allele frequencies in AD

TAP alleles	Patients with AD (n = 74)	Control subjects (n = 104)	Relative risk	Chi square	p Value
TAP1 A	63 (85%)	88 (85%)	1.04	0.0000	1.0000
B	10 (14%)	15 (14%)	0.93	0.0296	0.8633
C	1 (1%)	1 (1%)	1.41	0.0000	1.0000
TAP2 A	42 (57%)	46 (44%)	1.04	0.0000	1.0000
B	17 (23%)	35 (34%)	0.93	0.0296	0.8633
C	11 (15%)	10 (10%)	1.41	0.0000	1.0000
D	2 (4%)	1 (1%)	2.86	0.0892	0.7652
E	2 (1%)	11 (11%)	0.23	2.8821	0.0896
G	0 (0%)	1 (1%)	0.00	0.0000	1.0000

TABLE V. TAP phenotype frequencies in AD

TAP allele phenotypes	Patients with AD (n = 37)	Control subjects (n = 52)	Relative risk	Chi square	p Value
TAP1 A	36 (97%)	50 (96%)	1.44	0.0000	1.0000
B	9 (24%)	13 (25%)	0.96	0.0053	0.9419
C	1 (3%)	1 (2%)	1.42	0.0000	1.0000
D	0 (0%)	0 (0%)	1.40	—	1.0000
TAP2 A	32 (86%)	35 (67%)	1.44	0.0000	1.0000
B	15 (41%)	31 (60%)	0.96	0.0053	0.9419
C	10 (27%)	10 (19%)	1.42	0.0000	1.0000
D	2 (8%)	1 (2%)	2.91	0.0908	0.7632
E	2 (3%)	10 (19%)	0.24	2.4562	0.1171
G	0 (0%)	1 (2%)	0.00	0.0000	1.0000

association between TAP alleles and diseases. It is worthwhile to investigate the role of TAP genes in HLA class II-associated disease, even though TAP gene products are involved in HLA class I-restricted antigen processing. HLA class I gene

products may be involved in the development of HLA class II-associated disease. Patients with AD produced IgE antibodies against several environmental allergens through regulation by HLA class II-restricted CD4⁺ helper T cells, although CD8⁺

TABLE VI. Linkage disequilibrium between TAP alleles and HLA-DRB1/DQB1 alleles

1. DRB1*1302/DQB1*0604-TAP alleles in control subjects (*n* = 52)

TAP alleles	++	+-	-+	--	Chi square	<i>p</i> Value	Δ
TAP1 A	3	0	47	2	0.1273	0.7212	0.0057
B	0	3	13	36	1.0612	0.3029	-0.0086
C	0	3	1	48	0.1439	0.7044	-0.0006
TAP2 A	3	0	32	17	1.5464	0.2137	0.0167
B	0	3	31	18	4.6997	0.0302	-0.0285
C	1	2	8	41	0.5713	0.4497	0.0052
D	0	3	1	48	0.1439	0.7044	-0.0006
E	0	3	10	39	0.7580	0.3840	-0.0064
G	0	3	1	48	0.1439	0.7044	-0.0006

2. DRB1*1302/DQB1*0604-TAP alleles in patients with AD (*n* = 37)

TAP alleles	++	+-	-+	--	Chi square	<i>p</i> Value	Δ
TAP1 A	9	0	27	1	0.3304	0.5654	0.0214
B	4	5	5	23	2.6155	0.1058	0.0317
C	0	9	1	27	0.3304	0.5654	-0.0038
TAP2 A	9	0	23	5	1.8583	0.1728	0.0478
B	2	7	13	15	1.6556	0.1982	-0.0341
C	2	7	8	20	0.1392	0.7091	-0.0079
D	1	8	2	26	0.1439	0.7044	0.0044
E	1	8	0	28	3.1975	0.0738	0.0118

cytotoxic T lymphocytes predominantly infiltrate the skin lesions of active AD in the context of HLA class I-restricted antigen recognition.¹⁰ In addition, TAP transporters may be involved in HLA class II-restricted endogenous antigen processing.²⁴

Polymorphisms in rat and murine TAP genes have been shown to alter the spectrum of peptides bound to a major histocompatibility complex (MHC) class I molecule.²⁵ It is possible that differences in human TAP genes could also cause alteration in translocation of the peptides. Murine TAP molecules preferentially transport the peptides with hydrophobic C termini, although human TAP molecules translocate well those peptides with basic C termini and a broader range of peptides.²⁶ At least in human antigen processing pathways, the TAP molecule may play a role in selecting the size of peptides exclusively. Because the MHC class I gene in rats is less polymorphic, compared with human and murine MHC class I, one possible explanation is that rat TAP gene products may, in part, serve as a functional molecule of antigen recognition instead of MHC class I molecule. Furthermore, murine and human TAP molecules associate in the ER with MHC class I molecules. MHC class I heavy chain is complexed

with β_2 -microglobulin and the TAP1 subunit in the absence of TAP2. It ensures the colocalization of TAP and MHC class I in the ER and optimizes the exposure of MHC class I to peptide. MHC class I heavy chain associates with ER chaperone calnexin immediately after the synthesis.²⁷ Murine MHC class I heavy chain dissociates from calnexin before associating with β_2 -microglobulin and TAP.²⁸

In summary, functional discrepancies exist among human, mouse, and rat TAP molecules. Human TAP may exclusively select the size of peptides, and this would explain the lack of association with diseases.

Several functional analysis methods for peptide translocation have been reported, including radio-labeled peptide accumulation in microsomes, peptide binding to class I molecules in semi-permeabilized cells by streptolysin O, and radiolabeled glycosylated peptide capture with lectin-coated Sepharose (Pharmacia, Uppsala, Sweden).⁸ Further development of an analysis method is required to investigate peptide translocation.

In conclusion, we further determined the existence of TAP alleles in Japanese patients with AD. No association was observed with allele, amino acid residue frequencies. Further functional studies of the antigen processing pathway are needed

to clarify the precise antigen presentation mechanisms in the development of AD.

We thank Ms. Mitsuko Miyamoto for serologic HLA typing and Dr. Stephen H. Powis (Guy's Hospital, London) for his valuable discussion.

REFERENCES

1. Trowsdale J, Hanson I, Mockridge I, Beck S, Townsend A, Kelly A. Sequences encoded in the class II region of the MHC related to the 'ABC' superfamily of transporters. *Nature* 1990;348:741-4.
2. Deverson EV, Gow IR, Coadwell WJ, Monaco JJ, Butcher GW, Howard JC. MHC class II region encoding proteins related to the multidrug resistance family of transmembrane transporter. *Nature* 1990;348:738-41.
3. Spies T, Bresnahan M, Bahram S, et al. A gene in the human major histocompatibility complex class II region controlling the class I antigen presentation pathway. *Nature* 1990;348:744-7.
4. Monaco JJ, Cho S, Attaya M. Transport protein genes in the murine MHC: possible implications for antigen processing. *Science* 1990;250:1723-6.
5. Carrington M, Colonna M, Spies T, Stephens JC, Mann DL. Haplotypic variation of the transporter associated with antigen processing (TAP) genes and their extension of HLA class II region haplotypes. *Immunogenetics* 1993;37:266-73.
6. Powis SH, Tonks S, Mockridge I, Kelly AP, Bodmer JG, Trowsdale J. Alleles and haplotypes of the MHC-encoded ABC transporters TAP1 and TAP2. *Immunogenetics* 1993;37:373-80.
7. Powis SH, Rosenberg WMC, Hall M, et al. TAP1 and TAP2 polymorphism in coeliac disease. *Immunogenetics* 1993;38:345-50.
8. Hill A, Ploegh H. Getting the inside out: the transporter associated with antigen processing (TAP) and the presentation of viral antigen. *Proc Natl Acad Sci USA* 1995;92:341-3.
9. Kuwata S, Yanagisawa M, Saeki H, et al. Polymorphisms of transporter associated with antigen processing genes in atopic dermatitis. *J ALLERGY CLIN IMMUNOL* 1994;94:565-74.
10. Marsh DG, Zwollo P, Huang SK, Ghoush B, Ansari AA. Molecular studies of human response to allergens. *Cold Spring Harb Symp Quant Biol* 1989;54:459-70.
11. Saeki H, Kuwata S, Nakagawa H, et al. HLA and atopic dermatitis with high serum IgE levels. *J ALLERGY CLIN IMMUNOL* 1994;94:575-83.
12. Saeki H, Kuwata S, Nakagawa H, et al. Analysis of disease-associated amino acid epitopes on HLA class II molecules in atopic dermatitis. *J ALLERGY CLIN IMMUNOL* 1995;96:1061-8.
13. Saiki RK, Gelfand DH, Stoffel S, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 1988;239:487-91.
14. Beck S, Kelly A, Radley E, Khurshid F, Alderton RP, Trowsdale J. DNA sequence analysis of 66Kb of the human MHC class II region encoding a cluster of genes for antigen processing. *J Mol Biol* 1992;228:433-41.
15. Djilali SI, Caillat ZS, Schmitz J, et al. Polymorphism of antigen processing (TAP, LMP) and HLA class II genes in celiac disease. *Hum Immunol* 1994;40:8-16.
16. Bodmer JG, Marsh SG, Albert ED, et al. Nomenclature for factors of the HLA system, 1994. *Tissue Antigens* 1994;44:1-18.
17. Svejgaard A, Platz P, Ryder LP. HLA and disease 1982—a survey. *Immunol Rev* 1983;70:193-226.
18. Ronningen KS, Undlien DE, Ploski R, et al. Linkage disequilibrium between TAP2 variants and HLA class II alleles: no primary association between TAP2 variants and insulin-dependent diabetes mellitus. *Eur J Immunol* 1993;23:1050-6.
19. Bijvoet SM, Hayden MR. Mismatch PCR: a rapid method to screen for the Pro207→Leu mutation in the lipoprotein lipase (LPL) gene. *Hum Mol Genet* 1992;1:541.
20. Tighe MR, Hall MA, Cardi E, et al. Associations between alleles of the major histocompatibility complex-encoded ABC transporter gene TAP2, HLA class II alleles, and celiac disease susceptibility. *Hum Immunol* 1994;39:9-16.
21. Kellar-Wood HF, Powis SH, Gray J, et al. MHC-encoded TAP1 and TAP2 dimorphisms in multiple sclerosis. *Tissue Antigens* 1994;43:129-32.
22. Spurkland A, Knutsen I, Undlien DE, et al. No association of multiple sclerosis to alleles at the TAP2 locus. *Hum Immunol* 1994;39:299-301.
23. van Endert PM, Liblau RS, Patel SD, et al. Major histocompatibility complex-encoded antigen processing gene polymorphism in IDDM. *Diabetes* 1994;43:110-7.
24. Malnati MS, Marti M, LaVaute T, et al. Processing pathways for presentation of cytosolic antigen to MHC class II-restricted T cells. *Nature* 1992;357:702-4.
25. Powis SJ, Deverson EV, Coadwell WJ, et al. Effect of polymorphism of an MHC-linked transporter on the peptides assembled in a class I molecule. *Nature* 1992;357:211-5.
26. Momburg F, Roelse J, Hammerling GJ, et al. Peptide size selection by the major histocompatibility complex-encoded peptide transporter. *J Exp Med* 1994;179:1613-23.
27. Sugita M, Brenner MB. An unstable beta 2-microglobulin: major histocompatibility complex class I heavy chain intermediate dissociates from calnexin and then is stabilized by binding peptide. *J Exp Med* 1994;180:2163-72.
28. Androlewicz MJ, Ortmann B, van Endert PM, et al. Characteristics of peptide and major histocompatibility complex class I/beta 2-microglobulin binding to the transporters associated with antigen processing (TAP1 and TAP2). *Proc Natl Acad Sci USA* 1994;91:12716-20.