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Polymorphisms of transporter associated with antigen processing genes in atopic dermatitis

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*We investigated polymorphisms of transporter associated with antigen process (TAP) genes in atopic dermatitis. We developed a polymerase chain reaction–restriction fragment length polymorphism method for discriminating TAP alleles. Genomic DNA was obtained from 29 Japanese patients with atopic dermatitis and 35 control subjects. Dimorphic regions of TAP1 and TAP2 genes were amplified by polymerase chain reaction. Amplified products were digested with restriction endonucleases to determine TAP alleles: *Sau3A1* for TAP1 codon 333 (Ile-Val), *AccI* for TAP1 codon 637 (Asp-Gly), and *EcoRII* for TAP2 codon 687 (Gln-Stop). We observed four alleles for TAP1 and dimorphism for TAP2 codon 687. Six of 35 controls had the TAP1 D allele, which has been reported to be a rare allele in Caucasian populations. Gene frequency of TAP1 637Asp exhibited a tendency to increase in the patients with atopic dermatitis. TAP1 637Asp and TAP1 A alleles were estimated to constitute a haplotype with DRB1*1302-DQB1*0604 and DRB1*0803-DQB1*0601 in the Japanese population. Because TAP1 and TAP2 genes are located between HLA-DQB1 and -DPB1 loci, analysis of TAP gene polymorphisms will be useful for a better understanding of susceptibility loci in HLA class II-associated disease. (J ALLERGY CLIN IMMUNOL 1994;94:565-74.)*

Key words: TAP gene, HLA, PCR, PCR-RFLP, atopic dermatitis

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Patients with atopic dermatitis (AD) produced IgE against several environmental allergens.¹ CD4-positive helper T cells also play a role in B cell-mediated immunity in combination with HLA class II-restricted antigen recognition pathways. On the other hand, CD8-positive cytotoxic T lymphocytes predominantly infiltrate the skin lesions of active AD coupling with HLA class I-restricted antigen recognition. Both HLA class I and class II antigens are supposed to contribute to the pathogenesis of AD.

Antigenic peptides are presented to self-restricted cytotoxic T cells by the HLA class I gene products. These peptides are degraded from cy-

Abbreviations used

AD:	Atopic dermatitis
bp:	Base pair
IDDM:	Insulin-dependent diabetes mellitus
MHC:	Major histocompatibility complex
PCR:	Polymerase chain reaction
PCR-RFLP:	Polymerase chain reaction–restriction fragment length polymorphism
SSO:	Sequence-specific oligonucleotide
TAP:	Transporter associated with antigen processing

tosolic proteins and assembled with class I heavy and light chains (β 2-microglobulin) in the endoplasmic reticulum and are then transported to the cell surface.^{2,3} The antigen processing pathway is controlled by products of genes that are mapped within the HLA class I region. After cytosolic proteins are degraded by a proteasome-like structure, designated as the low molecular mass polypeptide complex, the peptides are actively transported across the endoplasmic reticulum membrane by two recently discovered transporter genes. Trowsdale et al.,⁴ Deverson et al.,⁵ Spies et al.,⁶ and Monaco et al.⁷ reported the cDNA sequences of two genes, now designated as transporter associated with antigen processing (TAP1 and TAP2). They exhibited homology to the adenosine triphosphate (ATP)-binding cassette (ABC) family of transporters, which include cystic fibrosis transmembrane conductance regulator and multidrug resistance genes. TAP genes encode a heterodimer molecule, which is responsible for translocation of intracellular peptides across the endoplasmic reticulum membrane where they bind to class I molecules.

TAP gene polymorphisms have been found since the discovery of the genes. Two dimorphic sites were found in TAP1, each of which encodes one of two possible amino acids.⁸ In TAP2 four dimorphic sites have been found.⁹ One is a silent change, and another is a transition that creates a premature stop codon. Each of the other remaining two sites encodes two possible amino acids. Different combinations of polymorphism at these sites constitute four possible alleles at TAP1 and eight possible alleles at TAP2.⁹ TAP gene polymorphisms are supposed to cause alterations in the peptide processing pathway.

TAP gene polymorphisms have been investigated in several HLA-associated diseases because the TAP gene is regarded as one of the candidates for disease susceptibility gene or one of the additional genes conferring the susceptibility. Ankylosing spondylitis,¹⁰ celiac disease,^{10,11} and insulin-dependent diabetes mellitus (IDDM)^{10,12} have been investigated. All reports so far have failed to find a significant association between TAP alleles and diseases.

In this study we investigated the TAP1 and TAP2 alleles in patients with AD. Because TAP alleles have so far been studied in established B lymphoblastoid cell lines or Caucasian populations,^{8,9} we attempted to determine whether any differences in the frequencies of TAP alleles exist between Caucasians and the Japanese population. Japanese patients with AD showed a tendency to increases in HLA-DR13/DQ6¹³; therefore, analysis of TAP genes will provide more precise insights into their susceptibility to this disorder. In this study we used the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method¹³⁻¹⁶ with restriction endonucleases to discriminate TAP alleles. Previous reports used sequence-specific oligonucleotide (SSO) probes,⁸ sequence-specific or allele-specific amplification,⁹ and single-strand conformation polymorphisms⁸ to identify TAP alleles. Analysis by PCR-RFLP will facilitate the determination of TAP alleles exactly and quickly. Because TAP genes are located in the HLA class II region between DPB1 and DQB1 loci, we also estimated extended HLA haplotypes, including TAP genes in the Japanese population.

METHODS

Patients

In this study we investigated 29 unrelated Japanese patients with AD who were outpatients in the Department of Dermatology at the University of Tokyo Hospital. The patient group consisted of 19 male and 10 female subjects, aged 11 to 42 years (mean $\pm$ SD, 25.0 $\pm$ 6.6 years). All patients manifested the severe clinical form of AD and showed high serum IgE levels in the range of 10,500 to 75,600 U/ml (mean $\pm$ SD, 26,500 $\pm$ 34,200 U/ml). Thirty-five Japanese persons with no evidence of atopic disease served as control subjects. Venous blood was drawn from each subject after informed consent was obtained.

HLA typing

Serological HLA typing was performed in all patients and control subjects by the standard lymphocyte microcytotoxicity test. HLA-DRB1 and -DQB1 alleles

were also determined with the PCR-SSO method. Primers and probes were based on guidelines from the Eleventh International Histocompatibility Workshop.¹⁷ Hybridization signals with digoxigenin-11-dUTP-labeled probes were obtained by chemiluminescence assay of hydrolysis of AMPPD (Boehringer Mannheim, Mannheim, Germany) with alkaline phosphatase-conjugated anti-digoxigenin antibody.¹⁸

Oligonucleotides

Oligonucleotides were synthesized by the methoxyphosphoramidite method on an Applied Biosystems 381A DNA Synthesizer (Applied Biosystems, Inc., Foster City, Calif.). We used two sets of primers for the TAP1 gene and one set of primers for the TAP2 gene. Nucleotide sequences of the primers were designed according to the report by Carrington et al.⁸ and are shown in Table I.

PCR

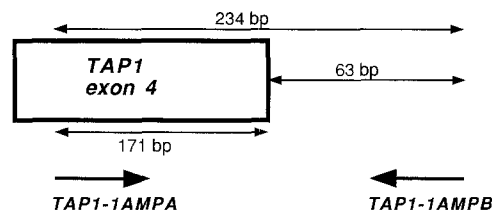
We amplified two regions of the TAP1 gene and one region of the TAP2 gene by PCR. We tentatively designated these regions TAP1-1, TAP1-2, and TAP2-2 regions, respectively. The TAP1-1 region contains a 234 base pair (bp) fragment spanning intron 4 and exon 4 of TAP1 gene including the dimorphic site at codon 333 (Ile3-Val). TAP1-2 region contains a 183 bp fragment spanning intron 9 and exon 10 of TAP1 gene including the dimorphic site at codon 637 (Asp-Gly). TAP2-2 region contains a 233 bp fragments spanning exon 11 and intron 11 of TAP2 gene including the dimorphic site at codon 687 (Stop-Gln). Alignments of primers and PCR-amplified fragments in TAP genes are shown in Fig. 1.

Genomic DNA was extracted from peripheral blood leucocytes by the standard phenol-chloroform-proteinase K method.¹⁹ PCR was carried out as described by Saiki et al.²⁰ 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 of each deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP), 10 pmol for each primer, and 0.4 U Ampli-Taq DNA polymerase (Perkin Elmer Cetus, Norwalk, Conn.). PCR cycles consisted of 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).

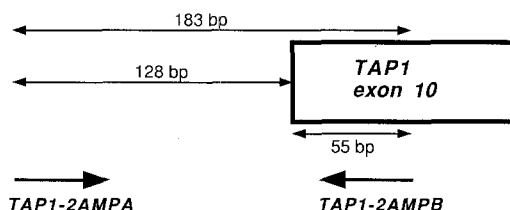
Direct sequencing of PCR products

We determined exact fragment sizes of the PCR products and the dimorphic sites by direct sequencing and compared the obtained sequences to those reported by Beck et al.²¹ PCR fragments were fractionated in 1.5% Nusieve GTG agarose gel (FMC BioProducts, Rockland, Md.) and purified. Direct sequencing was

TAP1-1 (TAP1 codon333)



TAP1-2 (TAP1 codon637)



TAP2-2 (TAP2 codon687)

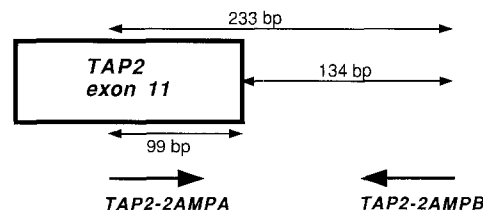


FIG. 1. Alignment of PCR primers and amplified fragments in TAP genes. PCR-amplified products contained intron sequences. Direct sequencing was performed, and obtained sequences were compared with genomic DNA sequences of TAP genes, as reported by Beck et al.²¹ TAP1-1 and TAP1-2 regions correspond to exon 4 and exon 10 of the TAP1 gene, respectively. The TAP2-2 region corresponds to exon 11 of the TAP2 gene.

performed by Ampli-Taq facilitated cycle sequencing reaction with the dideoxy terminator method (Applied Biosystems Inc.) 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 for PCR amplification.

Genotyping of dimorphisms of TAP1 and TAP2 by PCR-RFLP

Amplified PCR products were subjected to digestion with restriction endonucleases. Dimorphisms of TAP1

TABLE I. A panel of primers for TAP gene amplification

Primers	Nucleotide sequences
TAP1 at codon 333 (TAP1-1)	
TAP1-1AMPA:	5'-CACCTGACTCATTCTCT-3'
TAP1-1AMPB:	5'-ACTGACTCTGCCAAGTCT-3'
TAP1 at codon 637 (TAP1-2)	
TAP1-2AMPA:	5'-CCCTATCCAGCTACAACC-3'
TAP1-2AMPB:	5'-AACGCCACTGCCTGTCGCT-3'
TAP2 at codon 687 (TAP2-2)	
TAP2-2AMPA:	5'-ACAGTGCTGGTGATTGCTC-3'
TAP2-2AMPB:	5'-CACAGCTCTAGGGAAACTC-3'

Nucleotide sequences of the primers are derived from the report of Carrington et al.⁸

TABLE II. HLA-DR/DQ allele haplotype frequencies in AD

DR/DQ haplotype*		Patients with AD (n = 58)		Control subjects (n = 70)		Odds ratio	Chi square	p Value
DRB1	DQB1	No.	%	No.	%			
0101	0501	4	7	5	7	0.96	0.0000†	1.0000
0401	0301	0	0	1	1	0.00	0.0000†	1.0000
0403	0302	0	0	6	9	0.00	3.4738†	0.06234
0405	0401	2	3	7	10	0.32	1.2011†	0.2730
0406	0302	1	2	5	7	0.23	1.0481†	0.3059
0410	0402	4	7	1	1	5.11	1.2797†	0.2579
07	0201	1	2	0	0	3.68	0.008936†	0.9246
0802	0302	1	2	1	1	1.21	0.0000†	1.0000
0802	0402	2	3	2	3	1.21	0.0000†	1.0000
0803	0601	4	7	6	9	0.79	0.0004274†	0.9835
0901	0303	7	12	8	11	1.06	0.01257	0.9107
1101	0301	4	7	3	4	1.65	0.06565†	0.7977
1201	0301	0	0	2	3	0.00	0.3382†	0.5608
1201	0303	1	2	1	1	1.21	0.0000†	1.0000
1302	0604	5	9	2	3	3.20	1.0757†	0.2996
1401	0502	1	2	2	3	0.60	0.0000†	1.0000
1403	0301	3	5	1	1	3.76	0.4922†	0.4829
1405	0503	2	3	1	1	2.46	0.02723†	0.8689
1501	0602	4	7	7	10	0.67	0.09416†	0.7589
1502	0601	11	19	9	13	1.59	0.8977	0.3433
1602	0502	1	2	0	0	3.68	0.008936†	0.9246

*Common Japanese haplotypes between HLA-DRB1 and -DQB1 alleles were estimated according to the reports of the Eleventh International Histocompatibility Workshop.²⁴

†Chi square test with Yates' correction.

genes at codon 333 and at codon 637, included in the tentatively designated regions, TAP1-1 and TAP1-2, were detected by digestion with *Sau3A1* and *AccI*, respectively. Dimorphisms of the TAP2 gene at codon 687, included in designated region TAP2-2, was detected by digestion with *EcoRII*. Ten microliters of PCR-amplified products was digested with each enzyme for a total volume of 20 μ l. After the digestion reaction took place for 2 hours, samples were subjected to electrophoresis in 12% polyacrylamide gel in 1 $\times$ TBE buffer at 200 V. Digested fragments were visualized after staining with ethidium bromide.

Genotyping of TAP1 and TAP2 alleles

Four possible TAP1 alleles were determined in a combination of two dimorphic sites at codon 333 (Ile-Val) and codon 637 (Asp-Gly). As for TAP2 alleles, we classified eight possible TAP2 alleles into two groups according to the amino acid at the 687th position (Gln-Stop).

Estimation of haplotype including TAP alleles

Because TAP gene loci are located in the HLA class II region, we estimated haplotypes including HLA-

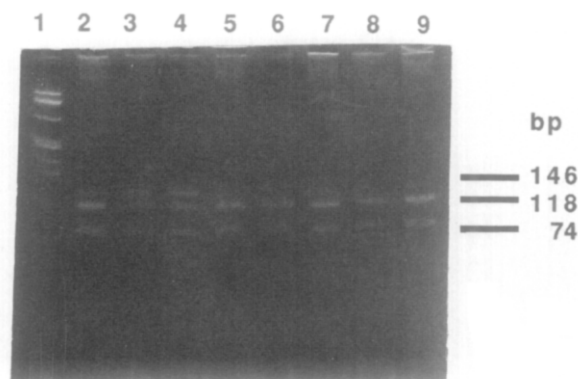


FIG. 2. Dimorphism of *Sau3AI* restriction fragments at codon 333 of *TAP1* gene. PCR products were digested with restriction endonuclease *Sau3AI* and electrophoresed in 12% polyacrylamide gel. Lane 1 contains DNA size marker, *HaeIII* digested ϕ 174. Lanes 2 to 9 contain samples from patients with AD. Individual subjects represented in lanes 1 to 3 are heterozygous for *TAP1* codon 333 (Val and Ile), and subjects represented in lanes 4 to 9 are homozygous for *TAP1* codon 333 (Ile). The PCR product with 234 bp was digested into 146 bp, 74 bp, and 14 bp for Val (GTC) or 118 bp, 74 bp, 28 bp, and 14 bp for Ile (ATC).

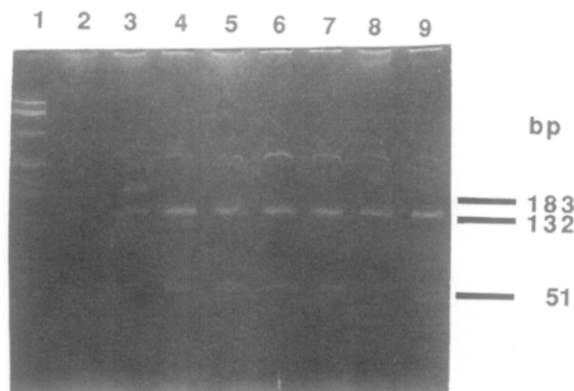


FIG. 3. Dimorphism of *AccI* restriction fragments at codon 637 of the *TAP1* gene. PCR products were digested with restriction endonuclease *Sau3AI* and electrophoresed in 12% polyacrylamide gel. Lane 1 contains DNA size marker, *HaeIII* digested ϕ 174. Lanes 2 to 9 contain samples from patients with AD. Individual subjects represented in lanes 1 and 2 are heterozygous for *TAP1* codon 637 (Gly and Asp), and subjects represented in lanes 3 to 9 are homozygous for *TAP1* codon 637 (Asp). The PCR product with 183 bp was digested into 132 bp and 51 bp for Asp (GAC) or remained uncut for Gly (GGC).

DRB1, -DQB1, and *TAP* genes in the patients and the control subjects. We compared the *TAP* alleles in patients with AD with HLA-DR/DQ-matched control subjects.

Nomenclature

Nomenclature for HLA specificities and HLA class II alleles was assigned according to guidelines from the World Health Organization Nomenclature Committee, HLA1991.²² Nomenclature for *TAP* alleles was assigned according to guidelines from the report of Powis et al.⁹

Statistics

Frequencies of each allele or epitope were compared with the chi-square test. The odds ratio of the disease was calculated according to the method of Svejgaard et al.²³ Statistical calculations were performed on a Macintosh IIfx personal computer with StatView software version 4.0 (Abacus Concepts, Berkeley, Calif.) with the version 7.1 system.

RESULTS

HLA specificities and HLA class II alleles in AD

Frequencies of serologic specificities were not significantly different between patients and control subjects. Frequency of DR13 showed a tendency to increase in patients. Five of 29 patients (17%) and two of 35 control subjects (6%) had DR13. The difference in DR13 frequency did not reach statis-

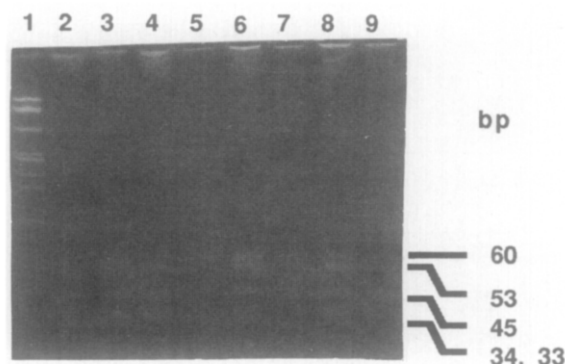


FIG. 4. Dimorphism of *EcoRII* restriction fragments at codon 687 of *TAP2* gene. PCR products were digested with restriction endonuclease *EcoRII* and electrophoresed in 12% polyacrylamide gel. Lane 1 contains DNA size marker, *HaeIII* digested ϕ 174. Lanes 2 to 9 contain the samples from patients with AD. Patients represented in lanes 2 to 9 are all homozygous for *TAP2* codon 687 (Stop). The PCR product with 233 bp was digested into 60 bp, 53 bp, 45 bp, 34 bp, 32 bp, and 9 bp for stop (TAG) or 53 bp, 45 bp, 34 bp, 33 bp, 32 bp, 27 bp, and 9 bp for Gln (CAG).

tical significance (odds ratio = 3.4), chi square with Yates' correction = 1.1417, $p = 0.2852$). HLA-DRB1 and -DQB1 allele frequencies were determined, and results are shown in Table II. Because DRB1 and DQB1 alleles were known to be in a strong linkage disequilibrium, we estimated DRB1 and DQB1 haplotypes, common in the

TABLE III. Amino acid residue frequencies in AD

		Phenotype frequency				
Amino acid residue		Patients with AD (n = 29)	Control subjects (n = 35)	Odds ratio	Chi square	p Value
TAP1	333 Ile	29 (100%)	35 (100%)	0.83	—	1.0000
	333 Val	8 (28%)	7 (20%)	1.52	0.5086	0.4757
	637 Asp	29 (100%)	35 (100%)	0.83	—	1.0000
	637 Gly	3 (10%)	13 (37%)	0.20	4.7290†	0.02965
TAP2	687 Gln	6 (21%)	8 (23%)	0.88	0.04359	0.8346
	687 Stop	29 (100%)	35 (100%)	0.83	—	1.0000

*Pc (corrected p value): p value was corrected with the number of alleles tested (n = 4 for TAP1).

†Chi square test with Yates' correction.

TABLE IV. Allele frequencies of TAP1 and TAP2 genes in AD

			Patients with AD (n = 58)		Control subjects (n = 70)		Odds ratio	Chi square	p Value
TAP1 alleles*	Codon		No.	%	No.	%			
A	Ile	Asp	52	90	57	81	1.98	1.6982	0.1925
B	Val	Gly	3	5	7	10	0.49	0.4655†	0.4950
C	Val	Asp	5	9	0	0	14.50	4.1931†	0.04058
D	Ile	Gly	0	0	6	9	0.00	3.4738†	0.06234

			Patients with AD (n = 58)		Control subjects (n = 70)		Odds ratio	Chi square	p Value
TAP2 alleles*	Codon		No.	%	No.	%			
A,C,D,E	Stop		52	90	62	89	1.34	0.03824	0.6232
B	Gln		6	10	8	11	0.89	0.03824	0.8449

*Nomenclature for TAP alleles are described according to the report of Powis et al.⁹

†Chi square test with Yates' correction.

A rare allele of TAP1 D was observed in six controls. All six individuals exhibited a combination of a heterozygosity at codon 333 (Ile and Val) and a homozygosity at codon 637 (Gly). Therefore TAP1 B (333Val-637Gly) and TAP1 D (333Ile-637Gly) were exclusively defined in these individuals. Statistical significance for TAP1 D disappeared after the p value was corrected by the number of alleles tested (n = 4). When an individual carried heterozygosities for both codon 333 and codon 637 of TAP1, two possible combinations of TAP1 A/TAP1 B or TAP1 C/TAP1 D were considered. However, a combination of TAP1 C/TAP1 D was estimated to be very rare, and we regard this as a combination of TAP1 A/TAP1 B as proposed by Powis et al.^{9,11}

Japanese population, according to the reports of the Eleventh International Histocompatibility Workshop.²⁴ DRB1*1302-DQB1*0604 and DRB1*1502-DQB1*0601 haplotypes showed a tendency to increase in the patients with AD, but no significance was indicated.

Direct sequencing of PCR-amplified TAP genes

We determined nucleotide sequences of PCR-amplified products by direct sequencing and confirmed them to be analogous to genomic DNA

sequences, as reported by Beck et al.²¹ PCR-amplified products of the TAP 1-1 region, including codon 333, encompassed a 234 bp fragment containing 171 bp of exon 4 and 63 bp of intron 4 in the TAP1 gene. PCR-amplified products of the TAP 1-2 region, including codon 637, encompassed a 183 bp fragment containing 128 bp of intron 9 and 55 bp of exon 10 in the TAP1 gene. PCR-amplified products of TAP 2-2, including codon 687, span the 233 bp fragment containing 99 bp of exon 11 and 134 bp of intron 11 in the TAP2 gene. These results are summarized in Fig. 2.

Gene frequency						
Amino acid residue		Patients with AD (n = 58)	Control subjects (n = 70)	Odds ratio	Chi square	p Value
TAP1	333 Ile	50 (86%)	64 (91%)	0.89	0.8878	0.3460
	333 Val	8 (14%)	6 (9%)	1.71	0.1174	0.7318
	637 Asp	55 (95%)	55 (79%)	5.00	5.6560†	0.01739
	637 Gly	3 (5%)	15 (21%)	0.20	5.6560†	0.01739
TAP2	687 Gln	6 (10%)	8 (11%)	0.89	0.03824	0.8449
	687 Stop	52 (90%)	62 (89%)	1.34	0.03824	0.6232

PCR-RFLP of TAP genes

We determined the expected size of the digested fragments by PCR-RFLP on the basis of the obtained nucleotide sequences of TAP genes. Sau3AI digestion of the TAP 1-1 region produced fragments of 146 bp, 74 bp, and 14 bp for codon 333 Val (GTC) or 118 bp, 74 bp, 28 bp, and 14 bp for codon 333 Ile (ATC). AccI digestion of TAP 1-2 region produced a 183 bp fragment for codon 637 Gly (GGC) or fragments of 132 bp and 51 bp for codon 637 Asp (GAC) (Fig. 3). EcoRII digestion of TAP 2-2 region produced fragments of 60 bp, 53 bp, 45 bp, 34 bp, 32 bp, and 9 bp for stop codon at codon 687 (TAG) or fragments of 53 bp, 45 bp, 34 bp, 33 bp, 32 bp, 27 bp, and 9 bp for codon 687 Gln (CAG) (Fig. 4). Representative results of PCR-RFLP are shown in Figs. 2, 3, and 4.

Amino acid residue frequencies of TAP molecules

We examined amino acid residue frequencies at each dimorphic site of TAP molecules, and the results are presented in Table III. Gene frequency of Asp at TAP1 codon 637 showed a tendency to increase in patients, whereas the significance disappeared after *p* values were corrected by multiplying by the number of epitopes tested. Phenotype frequencies of Gly at TAP1 codon 637 showed a tendency to decrease in patients with AD. We did not observe differences of TAP2 epitope frequencies that were dimorphic at TAP2 codon 687 (Stop-Gln) between patients and control subjects.

Allele frequencies of TAP gene

We also estimated TAP allele frequencies in patients with AD. Our estimation is shown in Table IV. When heterozygosities for both codon 333 Val/Ile and 637 Gly/Asp of TAP1 were observed in one subject, two possible allele combi-

nations of TAP1 A/TAP1 B or TAP1 C/TAP1 D were considered. Because a combination of TAP1 C/TAP1 D was thought to be very rare, we regarded this as a combination of TAP1 A/TAP1 B as proposed by Powis et al.^{9,11} The TAP1 D allele was observed in five of 58 total alleles in patients with AD. This allele has been thought to be rare in the Caucasian population. Because all subjects carried a TAP1 gene heterozygous at codon 333 (Val and Ile) and homozygous at codon 637 (Gly), we were able to determine that they have TAP1 B (333Val-637Gly) and D (333Ile-637Gly).

Estimation of haplotypes between HLA and TAP alleles

We attempted to determine whether TAP alleles were in linkage disequilibrium with DRB1/DQB1 alleles. The results are summarized in Table V. Seven class II haplotypes, common to Japanese persons, were selected, and we scrutinized the association between HLA alleles and TAP alleles. All four patients with AD and six control subjects with DRB1*0803-DQB1*0601 had 333Ile and 637Asp of TAP1 without exception; therefore, these 10 subjects were predetermined to have the TAP1 A allele. Five patients with AD and two control subjects with DRB1*1302-DQB1*0604 also had 333Ile and 637Asp of TAP1; therefore, these 10 subjects were also predetermined to have the TAP1 A allele. We were unable to find a possible linkage disequilibrium in the other remaining five HLA class II haplotypes: DRB1*0101-DQB1*0501, DRB1*0405-DQB1*0401, DRB1*0901-DQB1*0303, DRB1*1501-DQB1*0602, and DRB1*1502-DQB1*0601.

DISCUSSION

TAP gene polymorphisms so far have been investigated in several HLA-associated diseases in

TABLE V. Extended HLA haplotype including TAP1 gene in AD and control subjects

DRB1/DQB1 allele haplotype	TAP1 epitope				TAP1 allele			
	333I	333V	637D	637G	A	B	C	D
<i>DRB1*0101-DQB1*0501</i>								
AD (n = 4)	4 (2-4)	2 (2-0)	4	0	4 (2-4)	0	0	2 (2-0)
C (n = 5)	5 (4-5)	1 (1-0)	4 (4-3)	2 (1-2)	4 (3-4)	1 (0-1)	1	0
<i>DRB1*0405-DQB1*0401</i>								
AD (n = 1)	1 (1-0)	1 (0-1)	1 (0-1)	1 (1-0)	1 (1-0)	1 (0-1)	0	0
C (n = 7)	7 (6-7)	1 (1-0)	7 (3-7)	4 (4-0)	7 (3-7)	1 (1-0)	3 (3-0)	0
<i>DRB1*0803-DQB1*0601</i>								
AD (n = 4)	4	0 4	0	4	0 0	0	0	0
C (n = 6)	6	0 6	0	6	0 0	0	0	0
<i>DRB1*0901-DQB1*0303</i>								
AD (n = 7)	7 (4-7)	3 (3-0)	7 (5-7)	2 (2-0)	7 (4-7)	2 (2-0)	0	1 (1-0)
C (n = 8)	8 (4-8)	4 (4-0)	8 (4-8)	4 (4-0)	8 (4-8)	4 (4-0)	0	0
<i>DRB1*1302-DQB1*0604</i>								
AD (n = 5)	5 (4-5)	1 (1-0)	5	0	5 (4-5)	0	0	1 (1-0)
C (n = 2)	1	0 1	0	1	0 0	0		
<i>DRB1*1501-DQB1*0602</i>								
AD (n = 4)	4 (3-4)	1 (1-0)	4 (3-4)	1 (1-0)	4 (3-4)	1 (1-0)	0	0
C (n = 7)	7	0	7 (5-7)	2 (2-0)	7 (5-7)	0	2 (2-0)	0
<i>DRB1*1502-DQB1*0601</i>								
AD (n = 11)	11 (7-11)	4 (4-0)	11 (10-11)	1 (1-0)	11 (7-11)	1 (1-0)	0	3 (3-0)
C (n = 9)	9 (7-9)	2 (2-0)	8 (4-8)	5 (5-1)	8 (4-8)	2 (2-0)	2 (2-0)	0

We were not able to define TAP1 epitopes or TAP1 alleles when an individual carried haplotypes heterozygous at both HLA-DR/DQ and TAP alleles. Maximal number of TAP epitopes or TAP alleles is shown in this table. Range of numbers of epitopes or alleles is indicated in parentheses.

C, Control subjects.

Caucasian patients. Ankylosing spondylitis is one of the HLA class I-associated diseases but did not show a significant association with any of the TAP alleles,¹⁰ although this disease has been known to be strongly associated with HLA-B27. Among HLA class II-associated diseases, celiac disease^{10,11} and IDDM^{10,12} have been investigated and did not exhibit a significant association with TAP alleles. Each disease is associated with DQ2 and DR3/DR4, respectively, in Caucasian populations. Preliminary studies on membranous nephropathy²⁵ and IgA nephropathy²⁶ also demonstrated no significant associations. Each disease is associated with DR3 in Caucasians and DR4 in the Japanese, respectively. All reports so far, have failed to show a significant association between TAP alleles and specific diseases.

This study was undertaken to define the TAP1 and TAP2 alleles in patients with AD. Because the Japanese patients with AD had a tendency toward increases in frequency of HLA-DR13 a possible association with TAP alleles was investigated.¹³ We found that gene frequency of TAP1

637 Asp showed a tendency to increase in patients with AD, whereas TAP alleles were not increased in patients. In six of 35 controls, we observed TAP1 D allele, which has been previously reported to be a rare allele in Caucasian populations.⁹ As for TAP2 alleles in this study, we examined only codon 687, where a CAG(Gln) or TAG(Stop) transition was observed. TAP2 alleles with stop codon at 687 express a molecule shorter, by 17 amino acids than those with 687 Gln. 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 similar among different ethnic groups when we combined the data for Caucasians and homozygous typing cells with our results from the Japanese subjects.^{8,9,11}

Because TAP genes are located in the HLA class II region between DPB1 and DQB1 loci, we also estimated extended HLA haplotypes, including TAP genes in both patients and control subjects. DRB1*1302-DQB1*0604 and DRB1*0803-DQB1*0601 were estimated to constitute a hap-

lotype with TAP1 637 Asp epitope and TAP1 A allele. DRB1*1302-DQB1*0604 had a tendency to increase in the Japanese patients with AD, therefore, an increase of TAP1 637 Asp in our study may be due to an increase of this DR-DQ haplotype. In Caucasian populations, Powis et al.¹¹ reported possible sharing of haplotypes between TAP and HLA class II alleles. They observed such haplotypes as DR3-DQ2-TAP1A-TAP2A and DR7-DQ2-TAP1A-TAP2A. Carrington et al.⁸ also reported several extended haplotypes. They listed DRB1*0401-DQB1*0302-TAP2A, DRB1*1501-DQB1*0602-TAP1A, DRB1*0101-DQB1*0501-TAP2B, DRB1*1101-DQB1*0301-TAP1A, DRB1*07-DQB1*0201-TAP1A, and DRB1*0801-DQB1*0402-TAP2C. Ronningen et al.¹² also reported the estimation of linkage disequilibrium between TAP and HLA DR/DQ alleles in Caucasians. They found that TAP2 variants with 665 Thr and 687 stop codons were in linkage disequilibrium with both the DR4-DQ8 and DR3-DQ2 haplotypes, which are associated with IDDM. In contrast, the DR1-DQ5 and DR13-DQ6 haplotypes were associated with TAP2 665 Ala and 687 Gln variants.

Polymorphism in rat TAP genes has been shown to alter the spectrum of peptides bound to a major histocompatibility complex (MHC) class I molecule.²⁷ It is possible that differences in human TAP genes could cause an alteration in processing the peptides presented by HLA class I molecules, although no functional significance of these variations has been shown to date. Because the MHC class I gene in the rat is less polymorphic, compared with human and murine MHC class I, a possible explanation is that the rat TAP gene products may, in part, serve as a functional molecule for antigen recognition instead of an MHC class I molecule.²⁷

It is worthwhile to investigate the role of TAP genes in HLA class II-associated disease, even though TAP gene products are engaged in HLA class I-restricted antigen processing. HLA class I gene products may be involved in the development of HLA class II-associated disease. IDDM is associated with DR3/DR4 in Caucasian populations, and T cells infiltrating pancreatic β -islet cells are predominantly CD8-positive. Likewise, patients with AD produced IgE antibodies against several environmental allergens through regulation by HLA class II-restricted CD4-positive helper T cells, although CD8-positive cytotoxic T lymphocytes predominantly infiltrate in the skin

lesions of active AD in combination with HLA class I-restricted antigen recognition.¹ In addition, TAP may be involved in HLA class II-restricted endogenous antigen processing.²⁸

In this study we used the PCR-RFLP method with restriction endonucleases to discriminate TAP alleles. Carrington et al.⁸ used SSO probes and single-strand conformation polymorphism to discriminate TAP alleles.⁸ Powis et al.⁹ used sequence-specific or allele amplification, designated as an amplification refractory mutation system. We provided an alternative method for identifying TAP alleles. In extensive studies associated with the Eleventh International Histocompatibility Workshop, some SSO probes failed to exhibit their specificities. Inoko reported that PCR-RFLP, with restriction enzyme *AcyI*, was superior when compared with the SSO method for identifying the HLA-DQB1 fifty-seventh codon. They also reported a discrepancy at the HLA-DQA1 fifty-fifth codon between the SSO method and PCR-RFLP with *MboII*. We have also confirmed the benefit of the PCR-RFLP method for determining the HLA-DQB1 and -DPB1 alleles.^{15, 16} Allele-specific amplification is now being examined in other regions in the TAP2 gene, except those investigated in this study, in which restriction site is unavailable. 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.

In conclusion, we determined the frequency of TAP alleles in Japanese patients with AD. TAP1 637 Asp may contribute to the pathogenesis of AD in combination with HLA-DRB1*1302/DQB1*0604. Further in vitro study is needed to determine whether TAP molecules play an independently important role in the development of this disease, whether TAP molecules function as an additional factor in combination with classical HLA molecules, or whether the increased frequency of TAP alleles is merely due to the increase in extended HLA haplotypes.

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