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Alleles and haplotypes of the MHC-encoded ABC transporters *TAP1* and *TAP2*

Stephen H. Powis, Susan Tonks, Ian Mockridge, Adrian P. Kelly, Julia G. Bodmer, and John Trowsdale

Imperial Cancer Research Fund Laboratories, 44 Lincoln's Inn Fields, London, WC2A 3PX, UK

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Abstract. *TAP1* and *TAP2* are two major histocompatibility complex (MHC) genes, located between *HLA-DP* and *-DQ*, whose products form a transporter molecule involved in endogenous antigen processing. Polymorphic residues have been described in both genes and, in this study, we have identified another polymorphic site within the adenosine triphosphate (ATP)-binding domain of *TAP2*. We have used the amplification refractory mutation system (ARMS) polymerase chain reaction (PCR) to characterize *TAP1* and *TAP2* alleles and haplotypes in a reference panel of 115 homozygous typing cell lines. Of four possible *TAP1* alleles, we observed three, and of eight possible *TAP2* alleles, we observed five. Among 88 (homozygous typing cells) (HTCs) homozygous at *HLA-DR, -DQ* and *-DP*, 80 were also homozygous at *TAP1* and *TAP2*. Of 27 HTCs homozygous at *HLA-DR* and *-DQ*, but heterozygous at *-DP*, 14 were homozygous at *TAP1* or *TAP2* and 13 heterozygous, consistent with recombination taking place either side of the *TAP* loci. Of the fifteen possible combinations of *TAP1* and *TAP2* alleles, we observed eleven, each at a frequency similar to that predicted by individual allele frequencies. In this ethnically heterogeneous panel there is no indication that particular combinations of *TAP1* and *TAP2* have been maintained together.

Introduction

The class II region of the major histocompatibility complex (MHC) contains two γ-interferon (INF-γ) inducible genes, *TAP1* and *TAP2*, whose products are involved in endogenous antigen processing (Monaco et al. 1990; Deverson et al. 1990; Trowsdale et al. 1990; Spies et al. 1990; Bahram et al. 1991; Powis et al. 1992b). Both gene products are members of the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily, which includes proteins known to transport peptides across bacterial cell walls (Higgins et al. 1990). Cell lines which are defective in either gene exhibit abnormal intracellular class I assembly, low levels of class I at the cell surface, and fail to present defined class I restricted epitopes to cytotoxic T cells. These defects can be corrected by transfection with the appropriate wild-type *TAP1* or *TAP2* complementary DNA (cDNA; Spies and DeMars 1991; Powis et al. 1991; Kelly et al. 1992; Spies et al. 1992). These and other observations have led to the hypothesis that cytoplasmic peptides are transported into the endoplasmic reticulum by a complex of TAP1 and TAP2 (Robertson 1991).

Many human diseases are associated with the MHC, but because genes within the complex are in linkage disequilibrium it has not always been possible to assign susceptibility to individual genes (Tiwari and Terasaki 1985). Analysis of polymorphism within newly discovered MHC genes may further help to delineate extended haplotypes. *TAP1* and *TAP2* are of particular interest because their involvement in antigen presentation makes them candidate disease susceptibility genes. In rats, polymorphism within *TAP2* alters the spectrum of peptides bound by the class I allele *RT1.A*^{a} and its subsequent recognition by allogeneic T cells (Powis et al. 1992a). On the basis of this observation it has been suggested that *TAP1* and *TAP2* might be encoded within the MHC to permit specific coevolution between linked *HLA* and *TAP* alleles (Parham 1992).

We, and others, have recently described polymorphism within the coding regions of human *TAP1* and *TAP2* (Powis et al. 1992b; Colonna et al. 1992). In this

Correspondence to: S. H. Powis.

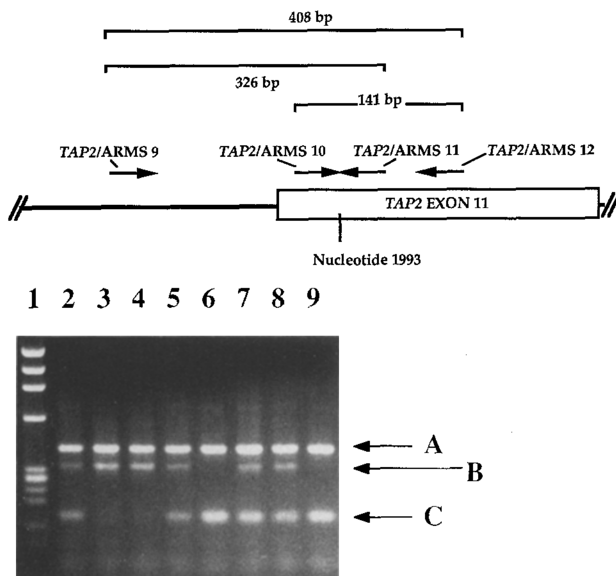

Fig. 1. Typing *TAP1* position 665 by ARMS PCR. Four oligonucleotides were included in each PCR reaction mix. Two of these, one sense (*TAP2*/ARMS10) and one anti-sense (*TAP2*/ARMS11), were designed such that their 3′ terminal nucleotides were complementary to one of the two variants of *TAP2* nucleotide 1993, which encodes amino-acid 665. *TAP2*/ARMS10 would only ampify efficiently if *TAP2* nucleotide 1993 was A, encoding Thr-665; *TAP2*/ARMS11 would only amplify efficiently if *TAP2* nucleotide 1993 was G, encoding Ala-665. The other two oligonucleotides, *TAP2*/ARMS9 (sense) and *TAP2*/ARMS12 (anti-sense), were complementary to flanking sequences located at assymetrical distances either side of nucleotide 1993. As illustrated, amplification resulted in a constant, control product of 408 bp, together with a single product of 326 bp if the sample was homozygous for Ala-665, a single product of 141 bp if the sample was homozygous for Thr-665, or both products if the sample was heterozygous. The products of eight amplified reaction mixes after electrophoresis in 2% agarose are shown. A – 408 bp control product, B – 326 bp Ala-665 specific product, C – 141 bp Thr-665 specific product. Lane 1 – molecular weight markers; lanes 2, 5, 7 and 8 – heterozygotes; lanes 3 and 4 – Ala-665 homozygotes; lanes 6 and 9 – Thr-665 homozygotes. Oigonucleotide sequences are shown in Table 1.

study, we report a further polymorphic site within the ATP-binding domain of *TAP2*. To analyze the alleles and haplotypic combinations of the *TAP* genes, we have used the amplification refractory mutation system (ARMS) polymerase chain reaction (PCR) to characterize the known *TAP1* and *TAP2* polymorphisms within a panel of 115 homozygous typing cell lines (HTCs).

Materials and methods

Isolation and sequencing of cDNA clones. cDNA libraries were constructed in a derivative of the CDM8 vector (Seed 1987). Libraries were screened with a full-length *TAP2* cDNA and secondary screening of positive clones was performed as previously described (Kendall et al. 1990). DNA from positive clones was prepared using standard protocols and sequenced by the dideoxynucleotide chain termination method. 20-mer oligonucleotides, complementary to *TAP2* at approximately 200 base pair (bp) intervals in both sense and anti-sense directions, were used as sequencing primers (Powis et al. 1992b).

DNA samples. Genomic DNA was prepared from homozygous typing cells (HTCs) used in the Tenth International Histocompatibility Workshop and from a panel of HTCs maintained by the Imperial Cancer Research Fund Tissue Antigen Laboratory. *HLA-DR, -DQ* and *-DP* types were obtained from published sources or were typed locally using Workshop sequence-specific oligonucleotide probes (Kimura et al. 1992).

PCR amplification. Genomic DNA samples (0.1 – 1 μg) were amplified in 100 μl reaction mixtures containing 0.25 μg of each oligonucleotide primer, 200 μM dNTPs, 1 × *Taq* DNA polymerase buffer, and 2 units of *Taq* DNA polymerase (Promega, Madison, WI), overlaid with mineral oil. Reaction conditions, using a thermal reactor (Hybaid, Teddington, England), were: 95° C for 5 min; 35 cycles of 94° C for 1 min, the appropriate annealing temperature for 2 min, 72° C for 2 min; 72° C for 10 min. Reaction products were separated on a 2% agarose gel and stained with ethidium bromide.

Results

Polymorphism within TAP1 and TAP2. Polymorphism within *TAP1* has been characterized by Colonna and co-workers (1992), who identified two polymorphic residues, Ile for Val at position 333 and Asp for Gly at position 637, after single-strand conformation polymorphism (SSCP) analysis of 21 HTCs. We have identified polymorphism within *TAP2* by sequencing clones from different cDNA libraries. Previously, we reported sequences of *TAP2* which differed by two amino acid substitutions: Glu for Stop at position 687 and Ala for Thr at position 665, and a silent nucleotide substitution, T for G at position 2091 (Powis et al. 1992b). In that study, we typed 36 HTCs and 54 Caucasoid controls at all three polymorphic sites, but found only two combinations, Thr-665, Stop-687, G-2091 and Ala-665, Gln-687, T-2091. A similar observation was made by Colonna and co-workers (1992). In this study, therefore, we have typed for variation at only one of these three sites, position 665. A third variable site within the protein sequence of *TAP2* was inferred from the cDNA sequence published by Bahram and co-workers (1991), which contained the substitution Ile for Val at position 379. We now report a further polymorphism within the ATP-binding cassette of *TAP2*, A for G at nucleotide position 1693, resulting in the substition of Thr for Ala at amino acid position 565. This was identified by sequencing a full-length cDNA isolated from a library made from the B lymphoblastoid cell line JY. Apart from this single substitution, the JY clone was identical to the previously published *TAP2* sequence *RING11A*, with Val-379, Thr-665, Stop-687, and G-2091 at the other polymorphic sites (Powis et al. 1992b).

POSSIBLE ALLELES

TAP1

333	637	NAME	FREQUENCY	
ILE	ASP	*TAP1A*	167/206	(81.1%)
VAL	GLY	*TAP 1B*	30/206	(14.5%)
ILE	GLY	*TAP1C*	9/206	(4.4%)
VAL	ASP		0	

TAP2

379	565	665	687	NAME	FREQUENCY	
VAL	ALA	THR	STOP	*TAP2A*	134/216	(62.0%)
VAL	ALA	ALA	GLN	*TAP2B*	55/216	(25.5%)
ILE	ALA	THR	STOP	*TAP2C*	16/216	(7.4%)
ILE	THR	THR	STOP	*TAP2D*	8/216	(3.7%)
VAL	THR	THR	STOP	*TAP2E*	3/216	(1.4%)
ILE	THR	ALA	GLN		0	
VAL	THR	ALA	GLN		0	
ILE	ALA	ALA	GLN		0	

Fig. 2. Alleles of *TAP1* and *TAP2*. The 4 possible *TAP1* and eight possible *TAP2* alleles are illustrated. The frequency at which each allele was observed is shown as a proportion of the total number of chromosomes in which alleles could definitely be assigned. Only those alleles observed have been named.

Typing TAP1 and TAP2 polymorphism by ARMS PCR. In order to type a large number of genomic DNA samples for *TAP1* and *TAP2* polymorphism, we developed a variation of ARMS PCR (Newton et al. 1989; Lo et al. 1991). This method utilizes the fact that oligonucleotides with a mismatched 3′ residue will not function as PCR primers under appropriate conditions, and permits rapid genotyping of any known mutation or polymorphism. Figure 1 illustrates how this technique was used to type *TAP2* position 665. To check the specificity of the technique, 36 cell lines were typed by both ARMS PCR and solid-phase PCR sequencing, as previously described (Powis et al. 1992b). The results obtained by each method were identical (data not shown). Details of the ARMS PCR oligonucleotides used in this study are shown in Table 1.

Alleles and haplotypes of TAP1 and TAP2 in a panel of HTCs. Possible alleles of *TAP1* and *TAP2*, based on known polymorphisms, are shown in Figure 2, together with the frequency at which they were observed in this study. Of four possible *TAP1* alleles, we observed three, which we have named *TAP1A, 1B,* and 1C. Of eight possible *TAP2* alleles, we observed five, named *TAP2A* to *2E*. We typed 88 HTCs homozygous at *HLA-DR, -DQ,* and *-DP*. Of these, the 80 listed in Table 2 were also homozygous at *TAP1* and *TAP2*. The eight

Table 1. Oligonucleotide primers used for ARMS PCR typing of *TAP1* and *TAP2*.

Polymorphic site	Name	Oligonucleotide sequence	Specificity	Product size
TAP1 Position 333	*TAP1*/ARMS1	5′-CCCTGCACTGAGATTTGCAGACCTCTGGAG-3′	5′ flanking sequence	Control – 533 bp
(58° C)*	*TAP1*/ARMS2	5′-GATCAGTGTCCCTCACCATGGTCACCCGGA-3′	*TAP1* Ile-333	Ile-333 specific – 241 bp
	TAP1/ARMS3	5′-GGGCAGAAGGAAAAGCAGAGGCAGGGTCAC-3′	*TAP1* Val-333	Val-333 specific – 351 bp
	TAP1/ARMS4	5′-ACCTGGGAACATGGACCACAGGGACAGGGT-3′	3′ flanking sequence	
TAP1 Position 637	*TAP1*/ARMS5	5′-CATCTTCCCAGAATCTCCCCTATCCAGCTA-3′	5′ flanking sequence	Control – 429 bp
(58° C)*	*TAP1*/ARMS6	5′-CATCTTGGCCCCTTTGCTCTGCAGAGGTACA-3′	*TAP1* Asp-637	Asp-637 specific – 307 bp
	TAP1/ARMS7	5′-ACCCCCTGACAGCTGGCTCCCAGCCTCCC-3′	*TAP1* Gly-637	Gly-637 specific – 180 bp
	TAP1/ARMS8	5′-TGGGGAGGCATCCAATGGAACTGGATTTGG-3′	3′ flanking sequence	
TAP2 Position 379	*TAP2*/ARMS1	5′-TTGGAGGGCTGCAGACCGTTCGCAGTTTTG-3′	5′ flanking sequence	Control – 427 bp
(58° C)*	*TAP2*/ARMS2	5′-GAGACCTGGAACGCGCCTTGTACCTGCGCG-3′	*TAP2* Val-379	Val-379 specific – 328 bp
	TAP2/ARMS3	5′-ACAACCACTCTGGTATCTTACCCTCCTGAT-3′	*TAP2* Ile-379	Ile-379 specific – 158 bp
	TAP2/ARMS4	5′-ACATAGCTCCCCACGCTCTCCTGGTAGATC-3′	3′ flanking sequence	
TAP2 Position 565	*TAP2*/ARMS5	5′-CTCACAGTATGAACACTGCTACCTGCACAG-3′	5′ flanking sequence	Control – 400 bp
(60° C)	*TAP2*/ARMS6	5′-TGTTCTCCGGTTCTGTGAGGAACAACAGTA-3′	*TAP2* Thr-565	Thr-565 specific – 161 bp
	TAP2/ARMS7	5′-ATCATCTTCGCAGCTCTGCAGCCCATAAAC-3′	*TAP2* Ala-565	Ala-565 specific – 298 bp
	TAP2/ARMS8	5′-GGAGCAAGCTTACAATTTGTAGAAGATACC-3′	3′ flanking sequence	
TAP2 Position 665	*TAP2*/ARMS9	5′-TTGGGGAATGGAATCCGGTGGTGTGAGGGC-3′	5′ flanking sequence	Control – 408 bp
(58° C)*	*TAP2*/ARMS10	5′-CAGTGCTGGTGATTGCTCACAGGCTGCAAA-3′	*TAP2* Thr-665	Thr-665 specific – 141 bp
	TAP2/ARMS11	5′-CACCAGGATCTGGTGGGCGCGCTGAACTAC-3′	*TAP2* Ala-665	Ala-665 specific – 326 bp
	TAP2/ARMS12	5′-TCAGCCGCTGCTGCACCAGGCGGGAATAGA-3′	3′ flanking sequence	

To analyze a single polymorphic site, four oligonucleotides are included in a single reaction mix. The expected sizes of the three ampified products (one constant, two variable) which can be produced in each reaction are listed in the final column; a schematic example for *TAP2* position 665 is illustrated in Figure 1a. The annealing temperature used in each reaction is indicated (*). In the oligonucleotide sequences, nucleotides *underlined once* represent potential mismatches, dependent upon allelic differences; nucleotides *underlined twice* are deliberate mismatches, introduced to enhance the specificity of ARMS PCR. Some of the oligonucleotides are complementary to intron sequence; the full genomic organization of *TAP1* and *TAP2* will be published elsewhere.

Table 2. Cell lines homozygous at *DR, DQ, TAP1, TAP2,* and *DP*.

Cell line	C*	XWS No.	DR serology	DQ	*DRB1*	*DQA1*	*DQB1*	*TAP1*	*TAP2*	*DPA1*	*DPB1*
KAS116	N	9003	1	5	*0101	*0101	*0501	1A	2A	*02	*1301
COX	Y	9022	3	2	*0301	*0501	*0201	1A	2A	*01	*0301
DUCAF	N	9019	3	2	*0301	*0501	*0201	1A	2A	*01	*0202
QBL	Y	9020	3	2	*0301	*0501	*0201	1A	2A	*01	*0202
VAVY	Y	9023	3	2	*0301	*0501	*0201	1A	2A	*02	*0101
EMH4	N		4	3	*04	*03		1A	2A		*0401
DEU	N	9025	4	7	*0401	*0301	*0301	1A	2A	*01	*0401
JHF	Y	9030	4	7	*0407	*0301	*0301	1A	2A	*01	*0301
600-SF			4	8				1A	2A		*0201
2046-SF			4	8		*03		1A	2A		*0401
BM14	Y	9033	4	8	*0401	*0301	*0302	1A	2A	*01	*0401
BOLETH	Y	9031	4	8	*0401	*0301	*0302	1A	2A	*01	*0401
BSM	Y	9032	4	8	*0401	*0301	*0302	1A	2A	*01	*0201
FLE			4	8		*03		1A	2A		*0401
LEIF-T	N		4	8				1A	2A		*0401
LKT2	N		4	8	*0406	*03		1A	2A		*0501
LKT17	N	9024	4	8	*0406	*03		1A	2A	*02	*0501
MT14B	Y	9098	4	8		*03		1A	2A		*0402
MTF			4	8		*03		1A	2A		*0402
YAR		9026	4	8	*0402	*0301	*0302	1A	2A	*01	*0401
JMF	Y		7	2		*0201		1A	2A		*0401
PITOT	Y	9051	7	2	*07	*0201	*0201	1A	2A	*01	*0401
T7526		9076	9	9	*0901	*0301	*0303	1A	2A	*0401	*1301
BM21	Y	9043	11	7	*1101	*0501	*0301	1A	2A	*02	*1001
JBUSH	Y	9035	11	7				1A	2A		*0401
JVM	Y	9039	11	7	*1102	*0501	*0301	1A	2A	*01	*0201
TISI	Y	9042	11	7	*1103	*0501	*0301	1A	2A	*01	*0402
HO301	N	9055	13	6	*1302	*0102	*0605	1A	2A	*02	*0501
HOR	N	9053	13	6		*0102		1A	2A		*0401
TEM	N	9057	14	5	*1401	*0101	*0503	1A	2A	*01	*0401
AZL	Y	9064	14	7	*1402	*0501	*0301	1A	2A	*01	*0402
LZL	Y	9099	14	7	*1402	*0501	*0301	1A	2A	*01	*0402
AKIBA			15	6	*1502	*0103	*0601	1A	2A	*0201	*0901
BBF	Y		15	6		*0102		1A	2A		*0401
LD2B	N	9083	15	6	*1501	*0102	*0602	1A	2A	*01	*0401
MST	N		15	6	*15	*0102		1A	2A		*0401
PGF	Y		15	6	*1501	*0102	*0602	1A	2A	*01	*0401
SCHU	N	9013	15	6	*1501	*0102	*0602	1A	2A	*01	*0402
TOKUNAGA			15	6	*1502	*0103	*0601	1A	2A	*02	*0901
WT18	Y		16	5			*0102	1A	2A	*01	*0301
AHB	Y		17	2		*0501		1A	2A		*0401
SC-TA			17	2		*0501		1A	2A		*0401
BERN-42			1	1		*0101		1A	2B		*0402
SA		9001	1	1				1A	2B		*0402
IBW4	Y		1	5	*0101	*0101		1A	2B		*0401
BON-CT	N		103	5	*0103	*0101		1A	2B		*0401
LKT3		9107	4	4	*0405	*0301	*0401	1A	2B	*02	*0501
SSTO			4	8	*0403	*03		1A	2B	*01	*0401
LBF	Y	9048	7	2	*0702	*0201	*0201	1A	2B	*02	*1701
DBB	Y	9052	7	9	*07	*0201	*03032	1A	2B	*01	*0401
TAB089	N	9066	8	6	*08031	*0103	*0601	1A	2B	*02	*0202
CB6B	Y	9060	13	6	*1301	*0103	*0603	1A	2B	*02021	*1901
HHKB	Y	9065	13	6	*1301	*0103	*0603	1A	2B	*01	*0401
BASILLIO			16	7		*0501		1A	2B		*0402
RML	Y		16	7	*1602	*05013	*0301	1A	2B	*01	*0402
WT49	Y		17	2	*0301	*0501	*0201	1A	2B		*0401
IBW9	Y	9049	7	2		*0201		1B	2A	*02	*0101
DKB	Y	9075	9	9	*09012	*03012	*03031	1B	2A	*01	*0401
KBB	Y		9	3	*09	*03		1B	2A	*01	*0401
BM16	Y	9038	12	7	*1201	*0501	*0301	1B	2A	*01	*0201

Table 2. (Continued).

Cell line	C*	XWS No.	DR serology	DQ	*DRB1*	*DQA1*	*DQB1*	*TAP1*	*TAP2*	*DPA1*	*DPB1*
OMW	N	9058	13	6	*1301	*0103	*0603	1B	2A	*02	*0101
SLE005	N	9059	13	6		*0102		1B	2A		*0301
AZH			16	5	*1601	*0102	*0502	1B	2A		*0301
CALOGERO	Y	9084	16	5		*0102		1B	2A		*0401
BTB	N	9067	8	4	*0801	*0401	*0402	1A	2C	*01	*0401
SPO010	Y	9036	11	5	*1101	*0102	*0502	1A	2C	*01	*0201
BM15	Y	9040	11	7	*1102	*0501	*0301	1A	2C	*01	*0301
BOB		9089	11	7	*1104	*0501	*0301	1A	2C	*01	*0402
WT48	Y		11	7				1A	2C		*0201
ABUC	Y	9061	14	5	*1401	*0101	*0503	1A	2C	*01	*0401
MGAR		9014	15	6	*1501	*0102	*0602	1A	2C	*01	*0401
JESTHOLM	Y	9004	1	5	*0101	*0101	*0501	1B	2B	*01	*0401
SPL	Y	9072	8	4	*08021	*0401	*0402	1B	2B	*01	*0402
BM9	Y	9068	8	4	*0801	*0401	*0402	1B	2D	*01	*0201
MADURA-T	Y	9069	8	4	*0801	*0401	*0402	1B	2D	*01	*0401
MCF	Y	9091	4	7	*0401	*0301	*0301	1A	2D	*01	*0402
BM92	Y	9092	4	8	*0404	*0301	*0302	1A	2E	*01	*0402
SAVC	Y	9034	4	8	*0401	*0301	*0302	1C	2A	*02	*1001
PLH	Y	9047	7	2	*07	*0201	*0201	1C	2C	*01	*1501
FPF	Y	9105	11	6	*11041	*0103	*0603	1C	2D	*02	*0201

C* Consanguineous, if known.

Table 3. Cell lines homozygous at *DR, DQ,* and *DP*, but heterozygous at *TAP1* or *TAP2*.

Cell line	C*	XWS No.	DR serology	DQ	*DRB1*	*DQA1*	*DQB1*	*TAP1*	*TAP2*	*DPA1*	*DPB1*
HOM2	N	9005	1	5	*0101	*0101	*0501	1A	(ILE,VAL)-379 (ALA, THR)-565 (ALA, THR)-665	*01	*0401
LOO81785	N	9018	3	2	*0301	*0501	*0201	(ILE,VAL)-333 (ASP, GLY)-637	2A	*01	*0301
LKT9			4	4	*04	*03		1A	(ILE, VAL)-379 ALA-565 (ALA, THR)-665	*01	*0201
SWEIG007	N	9037	11	7	*11011	*0501	*0301	1A	2A, 2B		*0402
WT50	N		11	7	*11	*0501		1A	2A, 2B		*0401
CBL			14	7		*0501		(ILE, VAL)-333 (ASP, GLY)-637	2A		*0402
WJRO76	N	9012	16	5				1A, 1C	2A		*02011
WT24	Y	9015	16	5				(ILE, VAL)-333 (ASP, GLY)-637	2A	*01	*0301

C* Consanguineous, if known.

cell lines heterozygous at *TAP1* and *TAP2* are listed in Table 3. Only one of these has been reported as consanguineous. We also typed 27 HTCs which were homozygous at *HLA-DR* and *-DQ*, but heterozygous at *-DP*. Fourteen were homozygous at *TAP1* or *TAP2* (Table 4), and 13 heterozygous (Table 5). Of the 15 possible allelic combinations of *TAP1* and *TAP2*, 11 were observed. The frequency at which each combination occurred was similar to that predicted from individual allele frequencies (Table 6).

Discussion

Polymorphism within *TAP1* and *TAP2* is of interest because both gene products are involved in the endo-

Table 4. Cell lines homozygous at *DR, DQ, TAP1* and *TAP2*, but heterozygous at *DP*.

Cell line	C*	XWS No.	DR serology	DQ	*DRB1*	*DQA1*	*DQB1*	*TAP1*	*TAP2*	*DPA1*	*DPB1*
EHM	N	9080	1	5	*0101	*0101	*0501	1A	2B	*01	*0401, *0402
LOUMAN-T			1	5		*0101		1A	2B		*0401, *0402
TER-ND	N		103	5	*0103	*0101		1A	2B	*01	*0201, *0401
MOR-ND	N		103	5		*0101		1A	2B		*0201, *0401
EJ32B	N	9085	3	2	*03	*0501		1A	2A		*0201, *0202
BER	N	9093	7	2	*07	*0201	*0201	1A	2B	*01	*0201, *0401
SCCA			7	9		*0201		1C	2B		*0201, *0401
LUY	N	9070	8	7	*0803	*0601	*0301	1A	2B	*01, *02	*0101, *0401
ARNT	N		13	6		*0103		1A	2A		*0201, *2001
EMJH	N	9097	13	6	*1302	*0102	*0604	1B	2A	*01	*0301, *0401
GPT	N		13	6		*0103		1A	2A		*0201, *0401
HAG			13	7	*1303	*0501		1B	2B		*0201, *0301
E4181324	N	9011	15	6	*1502	*0103	*0601	1A	2A	*01	*0201, *0401
LKT7			15	6		*0103		1A	2A		*0901, *1901

C* Consanguineous, if known.

Table 5. Cell lines homozygous at *DR* and *DQ*, but heterozygous at *TAP1, TAP2* and *DP*.

Cell line	C*	XWS No.	DR serology	DQ	*DRB1*	*DQA1*	*DQB1*	*TAP1*	*TAP2*	*DPA1*	*DPB1*
LWAGS		9079	1	5	*0102	*0101	*0501	1B	2A, 2B	*01	*0301, *0401
METTE	N		1	5	*0101	*0101		1A	(ILE, VAL)-379 ALA-565 (ALA, THR)-665		*0401, *0501
STEINLIN	N	9087	3	2	*0301	*0501	*0201	(ILE, VAL)-333 (ASP, GLY)-637	2A, 2B	*01	*0301, *0401
PRIESS	N		4	8		*03		1A	2A, 2B	*0101	*0301, *0401
SJAH			4	4		*03		1A	(ILE, VAL)-379 ALA-565 (ALA, THR)-665		*0201, *0501
CF996	N	9094	7	2	*07	*0201	*0201	(ILE, VAL)-333 (ASP, GLY)-637	2A, 2E	*01	*0401, *0402
BEI			7	9		*0201		(ILE, VAL)-333 (ASP, GLY)-637	2A, 2B		*0301, *0401
OLL	Y	9071	8	4	*08022	*0401	*0402	(ILE, VAL)-333 (ASP, GLY)-637	2A, 2B	*01	*0301, *0402
HID		9074	9	9	*09	*03		(ILE, VAL)-333 (ASP, GLY)-637	(ILE, VAL)-379 (ALA, THR)-565 THR-665	*01	*0201, *0501
LKT14	N	9103	9	9	*09	*03		(ILE, VAL)-333 (ASP, GLY)-637	(ILE, VAL)-379 (ALA, THR)-565 THR-665	*01, *02	*1601, *1901
BRIP	Y	9044	11	7		*0501		(ILE, VAL)-333 (ASP, GLY)-637	2A		*0201, *0402
BTID			18	4	*03	*0401		(ILE, VAL)-333 (ASP, GLY)-637	2A	*02	*0402, *1301
HERLUF	N		12	7	*1201	*0501		(ILE, VAL)-333 (ASP, GLY)-637	(ILE, VAL)-379 (ALA, THR)-565 (ALA, THR)-665	*01, *02	*0401

C* Consanguineous, if known.

Table 6. Observed haplotypes of *TAP1* and *TAP2*.

Possible haplotypes	Expected frequency	Observed frequency	
TAP1A TAP2A	50.3%	50.0%	(94/188)
TAP1A TAP2B	20.7%	21.3%	(40/188)
TAP1B TAP2A	9.0%	9.6%	(18/188)
TAP1A TAP2C	6.0%	7.4%	(14/188)
TAP1B TAP2B	3.7%	3.2%	(6/188)
TAP1B TAP2D	<1%	2.1%	(4/188)
TAP1A TAP2D	<1%	1.1%	(2/188)
TAP1A TAP2E	<1%	1.1%	(2/188)
TAP1C TAP2A	<1%	1.1%	(2/188)
TAP1C TAP2B	<1%	1.1%	(2/188)
TAP1C TAP2C	<1%	1.1%	(2/188)
TAP1C TAP2D	<1%	1.1%	(2/188)
TAP1B TAP2C	<1%	0	
TAP1B TAP2E	<1%	0	
TAP1C TAP2E	<1%	0	

Expected haplotype frequencies were determined by multiplication of the observed frequencies for each individual allele shown in Figure 2. Observed haplotype frequencies were derived from the HTCs homozygous at both *TAP1* and *TAP2* listed in Tables 2 and 4.

genous antigen-processing pathway (Spies and DeMars 1991; Powis et al. 1991; Kelly et al. 1992; Spies et al. 1992). In the rat, *TAP2*, and possibly *TAP1*, are functionally polymorphic (Powis et al. 1992 a). Similar observations within the human *TAP* genes would have obvious implications for HLA-associated disease. However, there are important differences between the rat and human systems. In the rat, alleles of *TAP2* differ by up to 25 amino acids, a degree of variation not observed in humans. Furthermore, compared to humans, the rat has a limited number of class I alleles. Thus, polymorphism within rat *TAP* genes may have evolved for a specific purpose not required in humans. If human *TAP* alleles do exist with high levels of variation comparable to the rat, they may be relatively rare.

ABC transporters typically consist of two hydrophobic domains and two ATP-binding domains (Higgins et al. 1990). In some members of the family, such as P-glycoprotein, all four domains are encoded in a single polypeptide. *TAP1* and *TAP2* each possess one hydrophobic domain and one ATP-binding domain, and may function as a heterodimer. Of the previously reported *TAP1* polymorphisms, one (position 333) is located within the hydrophobic domain and one (position 637) within the ATP-binding domain. Of the three *TAP2* polymorphisms, one (position 379) is located within the hydrophobic domain and two (positions 665 and 687) within the ATP-binding domain. In this study, we have identified a fourth *TAP2* polymorphic site (position 565) within the ATP-binding domain. In the rat, most *TAP2* variation occurs within the hydrophobic domain, and in P-glycoprotein, specificity of substrate transport can be altered by a mutation in this region (Safa et al. 1990). Conceivably, variation at either *TAP1* position 333 or *TAP2* position 379 may similarly alter specificity.

In order to characterize *TAP* polymorphism within a large number of samples, we have used a variation of ARMS PCR (Newton et al. 1989; Lo et al. 1991). ARMS PCR has advantages over other commonly used typing techniques such as sequence-specific oligonucleotide (SSO) typing. It requires neither radio-isotopes, digestion by restriction endonucleases, nor sequence analysis. Following PCR amplification, no further steps are required other than inspection after agarose gel electrophoresis. We have found its reliability and specificity comparable to other methods of genotyping. The ARMS PCR method we have described uses a product amplified by the two allele non-specific flanking oligonucleotides as an internal control (Fig. 1). Other techniques use oligonucleotides specific for a third-party gene, such as apolipoprotein B, to amplify a control product (Newton et al. 1989). We gain two advantages from our method. First, because the control product is amplified by oligonucleotides also used to amplify the allele specific product, loss of amplification by either flanking aligonucleotide (a potential false negative) will also result in loss of the control product. Such false negatives cannot be identified if a third-party gene is used as a control. Second, the genotype at one polymorphic site can be determined from a single reaction, whereas other methods require two reactions. Heterozygotes can be clearly distinguished from homozygotes of either allele (Fig. 1 a).

The main purpose of this study was to identify the alleles and haplotypes of *TAP1* and *TAP2* in a panel of HTCs. We observed three of four possible *TAP1* alleles, and five of eight possible *TAP2* alleles. It is possible that the other alleles shown in Figure 2 occur at low frequency, but were not observed in this study because the number of HTCs analyzed was insufficient. It is also possible that *TAP1* and *TAP2* contain other polymorphic sites which would further increase the number of potential alleles. Of the cDNA sequences which have been reported, two correspond to *TAP1A* (Trowsdale et al. 1990; Spies et al. 1990), one to *TAP2A* (Powis et al. 1992), one to *TAP2B* (Powis et al. 1992), and one to *TAP2E* (this paper). A fourth *TAP2* sequence, published by Bahram and co-workers (1991), constitutes a potential allele not observed in this study, Ile-379, Ala-565, Ala-665, Gln-697. However, as this sequence was obtained from two partial-length cDNAs, it may represent a mixture of alleles derived from both chromosomes of a heterozygous cell line.

As alleles at most *HLA* loci are in linkage disequilibrium, it will be interesting to determine whether linkage disequilibrium occurs between *TAP1* and *TAP2*, or between the *TAP* genes and other class II genes. Preliminary data has indicated linkage disequilibrium between *HLA-DQ* and *TAP2* in certain extended haplotypes (unpublished data), but because HTCs are derived

from diverse ethnic groups, we did not measure linkage disequilibrium in this study. We did, however, analyze a small number of HTCs in which homozygosity broke down between *HLA-DQ* and *-DP*, the region in which *TAP1* and *TAP2* are located. Within this panel, both homozygosity and heterozygosity at the *TAP* loci were observed, suggesting that recombination can occur between the *TAP* genes and *HLA-DP* or *HLA-DQ*. As *TAP1* and *TAP2* function as a complex, it could be reasoned that functionally important combinations of *TAP1* and *TAP2* alleles have been preferentially maintained together during evolution. Of the 15 possible combinations of *TAP1* and *TAP2*, we observed 11, occurring at frequencies similar to those predicted from the frequencies of individual alleles (Table 6). The combinations not observed were between infrequently occurring alleles, and their absence may simply reflect sample size. Because of the limitations inherent in an HTC panel, this data should be interpreted with caution, but it does suggest there is not widespread selection for particular combinations of *TAP1* and *TAP2*, and that recombination occurs between the two loci. We are currently typing *TAP1* and *TAP2* polymorphisms in a larger sample of Caucasoids to measure linkage disequilibrium involving the *TAP* loci.

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