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ORIGINAL ARTICLE

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Association of *TAP2* gene polymorphisms in Chinese patients with rheumatoid arthritis

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Abstract The aim of this study was to investigate the association between the polymorphism of transporters associated with antigen processing (*TAP1*/*TAP2*) genes and rheumatoid arthritis in Chinese patients. A total of 100 RA patients and 99 healthy control subjects were enrolled. Analyses with polymerase chain reaction (PCR) based restrictions were used to identify the polymorphisms of the *TAP1* and *TAP2* genes, which were mapped on chromosome 6. There was a significant difference in the distribution of the *TAP2* gene codon 565 polymorphism frequency between the RA patients and healthy control subjects ($p < 0.001$). The odds ratio for the risk of the 'A' allele in RA patients was 1.60 (95% CI: 0.82–2.92). No statistical associations in the distribution of the *TAP1* gene polymorphism frequency were found between RA patients and controls. There were some physical links found between *TAP1*/*TAP2* gene polymorphism loci. However, there was no linkage observed from *TAP1*/*TAP2* gene polymorphisms and HLA-DRB1*04 between RA patients and healthy controls. We concluded that the *TAP2* gene codon 565 'A' allele was associated with RA in Chinese patients in Taiwan. Individuals possessing the 'A' allele had a higher incidence of RA. A lack of association of *TAP1* gene polymorphisms between RA patients and healthy individuals was noted. The results of this study provide genetic evidence that *TAP2* gene codon 565 polymorphism may play a role in RA.

Keywords Rheumatoid arthritis · Single nucleotide polymorphism · *TAP1* and *TAP2* genes

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Abbreviations *MHC* Major histocompatibility · *MS* Multiple sclerosis · *PCR* Polymerase chain reaction · *RA* Rheumatoid arthritis · *SNP* Single nucleotide polymorphism

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting the synovial membranes of articulating joints [1]. There is evidence for immunopathogenesis in a genetic susceptible host [2, 3], but the genetic basis of RA is largely unknown. The major histocompatibility (MHC) class II region is an important susceptibility factor and the human leukocyte antigen (HLA)-DR4 has been associated with serious disease courses [4, 5]. The disease-associated HLA-DRB1*04 alleles (Dw4, Dw14) share a common sequence (amino acid position 70–74 of HVR3) or epitope [6]. Transporters associated with protein processing (*TAP*) genes are located within the class II region of the human HLA complex, between the DQ and DP loci [7]. *TAP1* and *TAP 2* polymorphisms have been identified in humans [8, 9, 10, 11]. If *TAP1* and *TAP2* genes occur within the HLA class II region and are polymorphic, *TAP* gene products may be important for RA immunopathogenesis from a functional point of view.

We used single nucleotide polymorphisms (SNP) as a tool to search for the genetic markers of RA [12, 13]. We hypothesized that the problems of whether *TAP1* and *TAP2* genes were associated with RA could be resolved using SNP. The AG polymorphisms at codon 333 and 637 of the *TAP1* gene and codons 379, 665 and 565 of the *TAP2* gene were previously reported by Kuwata et al. [14] Thus, the aim of this study was to investigate whether these polymorphisms were associated with RA in Chinese patients using polymerase chain reaction (PCR)-based restriction analysis. We compared genotype distributions and allelic frequencies in 100 patients with RA and 99 healthy individuals.

Materials and methods

We studied 100 Chinese patients with definite RA according to the 1987 revised American College of Rheumatology criteria [15]. The mean age of the patients was 52.2 ± 13.4 years and the mean age at disease onset was 45.2 ± 14.3 years. The mean duration of the disease was 7.0 ± 5.9 years. Sixty-one patients were seropositive RA; 48% of the RA patients were HLA-DRB1*04 positive. In addition, 99 unrelated healthy individuals living in central Taiwan served as controls. Informed consent was obtained from each individual who participated in the study (Table 1).

Genotyping and PCR for *TAP* genes

The genomic DNA was prepared from peripheral blood using a Genomaker DNA Extractor kit (Bloosm, Taiwan). Two sets of primers for the *TAP1* gene and three sets of primers for the *TAP2* gene were used. Nucleotide sequences of the primers are shown in Table 2. *TAP1* and *TAP2* genotyping was performed by the PCR-RFLP method using the specific primers shown in Table 2. Two dimorphic sites of *TAP1*–codons 333 and 637–were digested with DpnII and AccI, respectively. Three dimorphic sites of *TAP2* – codons 379, 665, 565 –were digested using AccII, MspI and RsaI, respectively (Table 3). PCR was carried out to a total volume of 50 μl, containing genomic DNA, 2–6 pmol of each primer, 1X Taq polymerase buffer (1.5 mM $MgCl_2$), and 0.25 units of AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA, USA). The PCR cycles consisted of an initial denaturation for 2 min at 94°C and 35 cycles of denaturation for 15 s at 94°C, annealing for 20 s at 60°C, and extension for 20 s at 72°C, which was followed by an extension of 10 min at 72°C. This cycling was performed in a programmable DNA Thermal Cycler (Perkin Elmer Cetus, Norwalk, Conn., USA). The PCR products were studied after restriction enzyme digestion (New England Biolabs, Beverly, Mass, USA). Then, 10 μl of the product was loaded into a 3% agarose gel containing ethidium bromide for electrophoresis.

Table 1 Basic data of RA patients and control subjects

	RA patients ($n=100$)	Controls ($n=99$)
Mean age (years)	52.2 ± 13.4	53.3 ± 8.9
Female	49.5 ± 13.5	53.4 ± 7.2
Male	60.5 ± 8.2	54.5 ± 11.2
Mean age at disease onset	45.2 ± 14.3	
Mean duration of disease	7.0 ± 5.9	
RF positive	61%	
DRB1*04 (+)	48 (48%)	27 (27.3%)

Table 3 Two dimorphic sites of*TAP 1*gene and three dimorphic sites of *TAP 2* gene

	Codon		RE	Allele size, bp
TAP1-1	333	ATC: Ile; GTC: Val	DpnII	A:118 + 74 + 28 + 15 G:146 + 74 + 15
TAP1-2	637	GAC: Asp; ATA: Gly	AccI	A:132 + 51 G:183
TAP2-1	379	GTA: Val; ATA: Ile	AccII	A:212 G:192 + 20
TAP2-2	665	ACA: Thr; GCA: Ala	MspI	A:207 + 20 G:227
TAP2-3	565	ACT: Thr; GCT: Ala	RsaI	A:132 + 23 G:155

HLA typing for DRB1*04

HLA-DRB1*04 typing was performed by PCR amplification with sequence-specific primers (RCR-SSP) described previously by Olerup [25].

Statistics

Allelic frequency was expressed as a percentage of the total number of alleles. Results from the control subjects and RA patients were compared using the χ^2 test (2×2 contingency tables) for statistical significance. When the assumption of the χ^2 test was violated, one cell had an expected count of <1 or $>20\%$ of the cells had an expected count of <5, Fisher's exact test was used. The distributions of the *TAP1* and *TAP2* gene polymorphisms in each group were evaluated. A p value less than 0.05 was considered statistically significant. The odds ratios (OR) were calculated from allelic frequency with 95% confidence interval (95% CI) for the polymorphisms of the *TAP* genes. Linkage disequilibrium between alleles was calculated with the standardized disequilibrium coefficient.

Results

The basic data between RA patients and healthy control subjects, including mean age and female/male ratio, were compared. The results are shown in Table 1. There were no significant differences between the RA patients and healthy controls in either age or gender.

The frequencies of the genotypes in the RA patients and control subjects are shown in Table 4. There were

Table 2 A panel of primers for amplification of TAP genes

Primers	Nucleotide sequences	Dimorphic sites
TAP 1 gene		***TAP 1***
TAP1-1AMPA:	5'-CACCCTGAGTGATTCTCT-3'	codon 333
TAP1-1AMPB:	5'-ACTGAGTCTGCCAAGTCT-3'	
TAP1–2AMPA:	5'-CCCTATCCAGCTACAACC-3'	codon 637
TAP1-2AMPB:	5'-AACGCCACTGCCTGTCGCT-3'	
TAP 2 gene		***TAP 2***
TAP2-1MAMPA*:	5'-CCGGTTCTGTGAGGAACAACAGT-3'	codon379
TAP2-1AMPB:	5'-GGAGCAAGCTTACAATTTGT-3'	
TAP2-2MAMPA*:	5'-GGTGATTGCTCACAGGCTGCCG-3'	codon 665
TAP2-2AMPB:	5'-CACAGCTCTAGGGAAACTC-3'	
TAP2-3MAMPA*:	5'-GAACGTGCCTTGTACCTGCGC-3'	codon 565
TAP2-3AMPB:	5'-ACCCCCAAGTGCAGCAC-3'	

Table 4 *TAP1* and *TAP2* genotype frequencies in RA patients and normal controls

Genotype		RA ($n=100$)	Controls ($n=99$)
TAP1–1	AA	64 (64%)	53 (53.5%)
	AG	34 (34%)	37 (37.4%)
	GG	2 (2%)	9 (9.1%)
TAP1–2	AA	76 (76%)	74 (74.7%)
	AG	22 (22%)	24 (24.2%)
	GG	2 (2%)	1 (1.0%)
TAP2–1	AA	6 (6%)	5 (5.1%)
	AG	41 (41%)	37 (37.4%)
	GG	53 (53%)	57 (57.6%)
TAP2–2	AA	9 (9%)	15 (15.2%)
	AG	46 (46%)	49 (49.5%)
	GG	45 (45%)	35 (35.4%)
TAP2–3*	AA	10 (10%)	0 (0%)
	AG	9 (9%)	19 (19.2%)
	GG	81 (81%)	80 (80.8%)

*$p < 0.001$ compared to controls by Fisher's exact test ($X^2 = 13.57$)

Table 5 Allelic frequency distribution of the *TAP1* and *TAP2* genes polymorphism in RA patients and normal controls

Genotype		RA ($n=100$)	Controls ($n=99$)
TAP1–1	A	162 (81%)	143 (72.2%)
	G	38 (19%)	55 (27.8%)
TAP1–2	A	174 (87%)	172 (86.9%)
	G	26 (13%)	26 (13.1%)
TAP2–1	A	53 (26.5%)	47 (23.7%)
	G	147 (73.5%)	151 (76.3%)
TAP2–2	A	64 (32%)	79 (39.9%)
	G	136 (68%)	119 (60.1%)
TAP2–3	A	29 (14.5%)	19 (9.6%)
	G	171 (85.5%)	179 (90.4%)

No significance of allelic frequencies between RA patients and control subjects

no significance genotype frequencies of the *TAP1* codons 333/637 and *TAP2* codons 379/665 between RA patients and normal controls. The genotype distributions of AG polymorphisms at *TAP2* codon 565 in the healthy subjects showed that 80 patients (80.8%) had the genotype GG and 19 patients (19.2%) had AG. Among the 100 RA patients, the genotype GG was found in 81 (81 %), AG in nine (9 %), and 10 patients (10%) had AA. There were no AA homozygotes in the healthy control group. There were significant statistical differences between the RA patients and the healthy subjects (χ^2 test, $p < 0.001$). The data were further subdivided into A and G groups according to the allelic frequencies in each group (Table 4). The allelic distribution of AG polymorphisms at the *TAP2* codon 565 in healthy subjects who had the 'A' allele was 0.096, and in those with the 'G' allele was 0.904. In the RA patients who had the 'A' allele the distribution was 0.145, and in those with the 'G' allele was 0.855. There were no significant statistical differences in allelic frequencies between patients and controls. The odds ratio for the risk of the 'A' allele in RA patients was 1.60, with a 95% CI ranging from 0.82 to 2.92.

The Hardy–Weinberg (HW) equilibrium test was performed and results showed that distributions of all genotypes were in HW equilibrium, except for the *TAP2–3* in RA patients ($p < 0.001$). The allelic and genotype frequencies of all SNP are shown in Tables 4 and 5. Linkage disequilibrium between alleles was calculated with the standardized disequilibrium coefficient. The results showed that there were linkages between *TAP1–1*(A)/*TAP1–2*(G) ($p < 0.01$), *TAP1–1*(G)/*TAP1–2*(G) ($p < 0.01$), *TAP 2–1*(A)/*TAP2–2*(A) ($p < 0.05$) in control subjects and *TAP2–1*(A)/*TAP2–2*(A) ($p < 0.01$), *TAP2–1*(A)/*TAP2–2*(G) ($p < 0.05$), *TAP 2–2*(A)/*TAP2–3*(A) ($p < 0.01$), *TAP2–2*(A)/TAP*2–3*(G) ($p < 0.01$) in patients (data not shown).

The associations of *TAP1* and *TAP2* alleles with HLA-DRB1*04 were estimated in RA patients and normal controls. The results are shown in Table 6. There were 48% of RA patients who carried HLA-DRB1*04. Only 27% of normal controls carried HLA-DRB1*04. No significance was observed in *TAP1* and *TAP2* gene polymorphisms between patients and controls. There was no linkage between HLA-DRB1*04 and *TAP1*/*TAP2* gene polymorphisms.

Table 6 TAP1 and TAP2 genotype frequencies in RA patients and normal controls between the positive and negative DRB1.04

Genotype		DRB1*04 (+)		DRB1*04 (-)	
		RA ($n=48$)	Controls ($n=27$)	RA ($n=52$)	Controls ($n=72$)
TAP1–1	AA	29 (60.4%)	15 (55.6%)	35 (67.3%)	38 (52.8%)
	AG	18 (37.5%)	11 (40.7%)	16 (30.8%)	26 (36.1%)
	GG	1 (20.8%)	1 (3.7%)	1 (1.9%)	8 (11.1%)
TAP1–2	AA	35 (72.9%)	20 (74.1%)	41 (78.9%)	4 (5.5%)
	AG	12 (25%)	7 (25.9%)	10 (19.2%)	28 (38.9%)
	GG	1 (2.1%)	0 (0)	1 (1.9%)	40 (55.6%)
TAP2–1	AA	3 (6.3%)	1 (3.7%)	3 (5.8%)	14 (19.4%)
	AG	27 (56.2%)	9 (33.3%)	14 (26.9%)	30 (41.7%)
	GG	18 (37.5%)	17 (63.0%)	35 (67.3%)	28 (38.9%)
TAP2–2	AA	4 (8.3%)	2 (7.4%)	5 (9.6%)	14 (19.4%)
	AG	16 (33.4%)	18 (66.7%)	30 (57.7%)	30 (41.7%)
	GG	28 (58.3%)	7 (25.9%)	17 (32.7%)	28 (38.9%)
TAP2–3	AA	7 (14.6%)	0 (0%)	3 (5.8%)	0 (0%)
	AG	4 (8.3%)	3 (12.5%)	5 (9.6%)	16 (10.7%)
	GG	37 (77.1%)	24 (87.5%)	44 (84.6%)	56 (89.3%)

No significance was observed

Discussion

TAP1 and *TAP2* genes located adjacent to the MHC class II region can control antigen presentation by class I molecules [16] and, as such, are candidate susceptibility genes for autoimmune diseases. Class I antigens may be important in the development of class II-associated disease, as for example in multiple sclerosis (MS). MS patients may have particular *TAP* alleles favoring the presentation of some viral or myelin autoantigen-driven peptides by HLA-class I molecules, which then initiates a strong T-cell mediated immune response leading to demyelination [17]. Defects in this process, mediated by transporter or MHC class I molecules, can have an influence on the T-cell repertoire, the response to diseases, and tolerance to self-antigens [18].

Polymorphisms in rat and murine *TAP* genes have been shown to alter the spectrum of peptides bound to MHC class I molecules [19]. It is possible that differences in human *TAP* genes could also cause alterations in translocation of the peptides. In human antigen-processing pathways the *TAP* molecule may play an exclusive role in selecting the size of peptides [20].

There has been little genetic evidence to support the hypothesis that *TAP1* and *TAP2* genes polymorphisms are associated in patients with RA. We investigated whether these polymorphisms were associated with Chinese patients with RA in Taiwan. The results demonstrated that only the prevalence of a variant of the *TAP2* gene codon 565 polymorphism was significantly higher in RA patients than in controls. However, there were no associations of *TAP1* gene codons 333 and 637, and *TAP2* gene codons 379 and 665 between RA patients and control subjects.

Rheumatoid arthritis is a progressive, destructive disease characterized by inflammation, abnormal immune responses and synovial hyperplasia. There is strong epidemiological evidence that genes contribute to the risk of developing many common diseases [17, 18]. Genes susceptible to rheumatic diseases are being proved using serial association studies by screening DNA polymorphisms for some genes such as IL-1β, IL-1 receptor antagonist [21] and vitamin D receptor genes [12, 13]. Evidence shows that many genes contribute to disease susceptibility and each may contribute a small increase in risk [22]. The *TAP2* gene codon 565 polymorphism was highly correlated with RA patients in our study. However, there are studies suggesting that the *TAP 2* gene polymorphism in RA patients was linked with HLA-DRB1*01 [23, 24]. We did find some linkage disequilibrium between *TAP1* and *TAP2* variants, but our results showed no significance in *TAP1* and *TAP2* gene polymorphisms between DRB1*04-positive RA patients and control subjects. So, despite the physical linkage displayed by these *TAP1/2* gene loci, they are not in linkage disequilibrium with HLA-DRB1*01. This evidence indicates that the *TAP2* gene codon 565 polymorphism may be a genetic marker for RA. The association of genes with RA could provide further risk identification, improved preventive medicine, and a choice of unique treatment strategies for some patients with subtypes of the disease. Therefore, using single nucleotide polymorphism (SNP) genotyping is likely to become a part of the routine management of patients with RA in the future.

In conclusion, we determined the existence of *TAP1* and *TAP2* gene alleles in Chinese patients with RA in Taiwan. No association was observed with allele amino acid residue frequencies except *TAP2* codon 565. There was no linkage with HLA-DRB1*01 in either RA patients or control subjects. Further functional studies of the antigen-processing pathway are needed to clarify the precise antigen-presentation mechanisms involved in the development of RA.

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