Analysis of the modifying effects of TAP 1/2 genes on cystic fibrosis phenotype

Filiz Özbaş-Gerçeker[1], Uğur Özçelik[2], Nural Kiper[2], Deniz Anadol[2], Ayhan Göçmen[2] Engin Yılmaz[3], Hayat Erdem-Yurter[3], Meral Özgüç[1,3]

[1]TUBITAK DNA/Cell Bank and Gene Research Laboratory, Institute of Child Health and Departments of [2]Pediatrics, and [3]Medical Biology, Faculty of Medicine, Hacettepe University, Ankara, Turkey

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Phenotypic variability has been reported in cystic fibrosis (CF) patients. TAP1 and TAP2 genes are encoding "the transporter associated with antigen processing" proteins. The aim of the present study was to analyze the frequency of TAP 1/2 variants in the Turkish population and to investigate a possible modifying role of these variants in CF phenotype. Sixty-three CF patients of known genotypes and 100 healthy control subjects were analyzed. There was a significant difference in the frequencies at positions 333 and 637 of TAP 1 gene and at position 665 of TAP 2 gene between patients and controls. Comparison of TAP gene polymorphisms in 36 CF patients homozygous for ΔF508 mutation with control subjects revealed a significant difference at position 665 of TAP 2 gene. These findings may be useful to assess the predisposition and to predict severity of the disease. We demonstrated that TAP genes might have modifying effects on the CF phenotype.

Key words: cystic fibrosis, TAP 1/2 genes, modifying genes.

Cystic fibrosis (CF) is an autosomal recessive genetic disorder with a prevalence of 1/2500 in the Caucasian population[1]. The disease manifests itself by pulmonary and pancreatic exocrine insufficiency, meconium ileus and pseudomonas infections. The mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) are known to be responsible for the disease[2]. These mutations may result in the lack of protein production, defective protein processing, defective regulation or in defective ion conduction[3]. CFTR was the first gene cloned by positional cloning[4] and the second chloride channel cloned[5]. CFTR gene was mapped to 7q31.2 and protein functions as a phosphorylation and nucleotide regulated small-conductance chloride channel[6], has been but is also regulates the epithelial sodium channels[7].

Phenotypic variability has been reported in CF patients[8]. It is known that CF patients are susceptible to different types of lung infections with some specific agents. Pier et al.[9] showed that CF patients were susceptible to Pseudomonas aeruginosa lung infection, and they reported that CFTR protein might have a host-defense function in the clearance of Pseudomonas aeruginosa from the respiratory tract. Pseudomonas aeruginosa lung infection primarily leads to a sustained immune response causing chronic inflammation and gradual tissue destruction resulting in premature death from respiratory insufficiency in CF patients[10]. However, the course of the disease differs between patients even if they have the same CFTR mutations. Mannose binding Lectin (MBL), a key factor in innate immunity which is thought to be associated with recurrent infections[11], and variant alleles in CF patients were analyzed and it was shown that lung function was significantly reduced in carriers of these variant alleles when compared to normal homozygotes. Therefore MBL deficiency might be a risk factor for CF patients[12]. Later it was concluded that CF and MBL deficiency results in a risk for Burkholderia cepacia colonization[13].

There may be other genes having modifying or modulating effects on the CF phenotype. TAP1 and TAP2 genes are such candidates located in the MHC class II region at 6p21.3 and encoding "the transporter associated with antigen processing" proteins[14]. TAP1 and TAP2 are half transporters[15] and they function in the ATP dependent transport of cytoplasmic peptides, which are generated from the degradation of cytoplasmic proteins by proteosome complexes, into the endoplasmic reticulum lumen[16]. TAP proteins are members of ATP-binding cassette (ABC) family of membrane translocators, which also include the CFTR[17].

Polymorphisms at positions 333 and 637[18], and 659[19] of TAP1 gene at 253[20], 379, 665 and 687[18], 565[21], and 577[22] of TAP2 gene have been reported. Some rare TAP2 polymorphisms were also reported in African populations[23]. It is thought that polymorphic variants of TAP genes can restrict the antigenic peptides that are bound and presented[24]. Individual variability in structure or expression of these genes can affect specificity and result in different sets of peptides derived from the same antigen being presented to T cells in different people, therefore TAP genes are attractive candidates of susceptibility/severity factors[25].

An association between TAP gene polymorphisms and many human diseases such as Graves' disease[26], rheumatoid arthritis[25], diffuse panbronchiolitis[27], hepatitis C virus infection[28], nickel allergy[29], juvenile onset psoriasis[30] and sarcoidosis[31] has been reported. The aim of the present study was to analyze the frequency of TAP1/2 variants in a healthy Turkish population and also to investigate a possible modifying role of these variants in the CF phenotype.

Material and Methods

Patients and Control Subjects

Sixty-three CF patients (mean age: 7.35, range 2-13 years, 31 male/32 female) of known genotypes were chosen from the total 443 CF patients followed at Hacettepe University Children's Hospital. Diagnosis of CF was established on the basis of abnormal sweat electrolytes and clinical features. Of the patients, 95.2% had pancreatic insufficiency and 17.5% were chronically infected, at least by one microorganism (S. aureus, P. aeruginosa) in the lower respiratory tract. In all CF patient, diagnosis was confirmed by mutation analysis. Thirty-six were homozygous for ΔF508 mutation. Eleven were homozygous for other CFTR mutations, and the remaining 16 patients were compound heterozygous.

One hundred unrelated healthy subjects were selected as a control group. Informed consent was obtained from the parents of the children and control subjects.

Genomic DNA Isolation

Blood samples from CF patients and control subjects were collected into EDTA tubes with verbal and written explanation and signed consent. Genomic DNA was isolated by standard salting out procedure[32]. The DNA concentrations were measured and DNAs were stored at –80°C until use.

Analysis of TAP Gene Polymorphisms and TAP Genotyping

The amplification refractory mutation system (ARMS)-polymerase chain reaction (PCR)[23] was used to determine TAP1 and TAP2 polymorphisms. Two polymorphisms in the TAP1 gene (positions 333 and 637) and three polymorphisms in the TAP2 gene (position 379, 565 and 665) were analyzed in this study. The oligonucleotide primer sequences were as previously described[23]. Genomic DNA samples (0.25 μg) were amplified in 25 μl reaction mixtures containing 0.25 μg of each oligonucleotide primer, 200 μM dNTP's, 1Xtaq DNA polymerase buffer and 0.5 units of Taq DNA polymerase (Promega, Madison, USA) overlaid with mineral oil. Reaction conditions using a thermal cycler (MJ Research PTC-200, Massachusetts, USA) 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 and 72°C for 10 min. Reaction products were separated on a 2% agarose gel containing 0.1% w/v ethidium bromide for visualization.

Flanking primers of each polymorphic site were used to have an "internal control" for each specific reaction. The variant amino acids for each position and the sizes of the resultant products are given in Table I.

Statistical Analysis

Since TAP genes can be found as heterozygous at more than one position, genotype and amino acid frequencies rather than allele or haplotype

frequencies were preferred. Both genotype and amino acid frequencies were calculated for CF patients and control subjects. Amino acid frequencies were compared using 2x2 chi-square test with df of 1, and genotype frequencies were compared by 3x2 chi-square test with df of 2 using the statistical program Stats version 1.1 (Decision Analyst Inc., Arlington).

Table I. The TAP variant amino acids for each position and the sizes of the ARMS-PCR products

Position	Control	Variant 1		Variant 2	
TAP 1-333	533 bp	Ile	241 bp	Val	351 bp
TAP 1-637	429 bp	Gly	180 bp	Asp	307 bp
TAP 2-379	427 bp	Ile	158 bp	Val	328 bp
TAP 2-565	400 bp	Thr	161 bp	Ala	298 bp
TAP 2-665	408 bp	Thr	141 bp	Ala	326 bp

ARMS-PCR: amplification refractory mutation system-polymerase chain reaction.

Results

The phenotype and genotype frequencies of all polymorphisms calculated for CF patients and control subjects are shown in Table II together with the χ^2 and P values. Statistically meaningful differences between Turkish CF patients and control subjects were found at TAP 1 positions 333 and 637 and TAP 2 position 665.

For TAP 1, at position 333 there was a significant increase in Val frequency in CF patients (χ^2=4.75, df: 1, p=0.046). The frequency of Ile and genotypes were not significantly different between the two groups.

At position 637, the frequency of Gly was significantly increased in CF patients (χ^2=4.08,

Table II. Comparison of TAP 1 and TAP 2 polymorphisms in Turkish control subjects and cystic fibrosis patients of known genotypes

	Control subjects		CF Patients			
TAP polymorphism frequencies	(n=100)	(%)	(n=63)	(%)	χ^2	P
TAP1 P333						
Phenotypes						
Ile	96	96	59	93.6	NS	
Val	25	25	26	41.3	4.75	0.046
Genotypes						
Ile/Ile	75	75	37	58.7		
Ile/Val	21	21	22	34.9	NS	
Val/Val	4	4	4	6.3		
TAP1 P637						
Phenotypes						
Asp	86	86	49	77.7	NS	
Gly	18	18	20	31.7	4.08	0.046
Genotypes						
Asp/Asp	82	82	43	68.2		
Asp/Gly	4	4	6	9.5	NS	
Gly/Gly	14	14	14	22.2		
TAP 2 P379						
Phenotypes						
Ile	26	26	16	25.4	NS	
Val	75	75	49	77.7	NS	
Genotypes						
Ile/Ile	25	25	14	22.2		
Ile/Val	1	1	2	3.2	NS	
Val/Val	74	74	47	74.6		

Table II. Continue next page.

Table II. Continued
Comparison of TAP 1 and TAP 2 polymorphisms in Turkish control subjects and cystic fibrosis patients of known genotypes

TAP polymorphism frequencies	Control subjects (n=100)	(%)	CF Patients (n=63)	(%)	χ^2	P
TAP 2 P565						
Phenotypes						
Thr	5	5	6	9.5	NS	
Ala	99	99	62	98.4	NS	
Genotypes						
Thr/Thr	1	1	1	1.6		
Thr/Ala	4	4	5	7.9	NS	
Ala/Ala	95	95	57	90.5		
TAP 2 P665						
Phenotypes						
Thr	100	100	60	95.2	4.85	0.046
Ala	1	1	9	14.3	11.8	0.001
Genotypes						
Thr/Thr	99	99	54	85.7		
Thr/Ala	1	1	6	9.5	12.03	0.003
Ala/Ala	0	0	3	4.7		

NS: not significant.

df: 1, p=0.046). The overall genotype frequency was not significantly different at this position.

When we examined phenotype frequencies of TAP 2 variants, no significant difference was observed in amino acid and genotype frequencies at positions 379 and 565; however, a significant difference was seen at position 665. Thr variant frequency at position 665 was significantly reduced in CF patients (χ^2=4.85, df: 1, p=0.046) but Ala frequency was increased (χ^2=11.8, df: 1, p=0.001). Comparison of the overall genotype frequencies at position 665 indicated a significant difference between CF patients and control subjects (χ^2=12.03, df: 2, p=0.003). Thr/Thr genotype was underrepresented while Thr/Ala and Ala/Ala genotypes were over represented in CF patients.

Furthermore, we analyzed homozygous patients with the genotype ΔF508/ΔF508. The comparison of these patients with control subjects revealed a significant difference only at position 665. The frequency of Ala was found to be higher in ΔF508/ΔF508 CF patients than the control subjects (χ^2=7.64, df: 1, p=0.008). When we compared the overall genotype frequencies in the two groups, a significant difference was observed (χ^2=7.89, df: 2,

Table III. Comparison of TAP 1 and TAP 2 polymorphisms in control subjects and ΔF508/ΔF508 CF patients

TAP polymorphism frequencies	Control subjects (n=100)	(%)	ΔF508/ΔF508 CF patients (n=63)	(%)	χ^2	P
TAP1 P333						
Phenotypes						
Ile	96	96	33	91.6	NS	
Val	25	25	15	40.6	NS	
Genotypes						
Ile/Ile	75	75	21	58.3		
Ile/Val	21	21	12	33.3	NS	
Val/Val	4	4	3	8.3		

Table III. continue next page.

Table III. Continued
Comparison of TAP 1 and TAP 2 polymorphisms in control subjects and ΔF508/ΔF508 CF patients

TAP polymorphism frequencies	Control subjects (n=100)	(%)	ΔF508/ΔF508 CF patients (n=63)	(%)	χ^2	P
TAP1 P637						
Phenotypes						
Asp	86	86	28	77.7	NS	
Gly	18	18	10	27.7	NS	
Genotypes						
Asp/Asp	82	82	26	72.2		
Asp/Gly	4	4	2	5.5	NS	
Gly/Gly	14	14	8	22.2		
TAP 2 P379						
Phenotypes						
Ile	26	26	8	22.2	NS	
Val	75	75	29	80.5	NS	
Genotypes						
Ile/Ile	25	25	7	19.4		
Ile/Val	1	1	1	2.7	NS	
Val/Val	74	74	28	77.7		
TAP 2 P565						
Phenotypes						
Thr	5	5	5	20.0	NS	
Ala	99	99	35	100	NS	
Genotypes						
Thr/Thr	1	1	1	2.7		
Thr/Ala	4	4	4	11.1	NS	
Ala/Ala	95	95	31	86.1		
TAP 2 P665						
Phenotypes						
Thr	100	100	35	97.2	NS	
Ala	1	1	4	11.1	7.64	0.008
Genotypes						
Thr/Thr	99	99	32	88.8		
Thr/Ala	1	1	3	8.3	7.89	0.031
Ala/Ala	0	0	1	2.7		

NS: not significant.

p=0.031). Thr/Thr genotype was underrepresented ΔF508/ΔF508 CF patients while Thr/Ala and Ala/Ala were overrepresented (Table III).

Discussion

In the present study, the frequencies of polymorphic variants of TAP genes were analyzed for a healthy Turkish population and CF patients. The frequencies of TAP gene polymorphisms differ among different populations. Table IV shows frequencies of TAP variants in several representative populations. It can be seen that the Turkish population has a very different frequency distribution than the others. The most striking observation was the underrepresentation of the Ala variant at position 665: 1% in the Turkish population versus 46.9% in Polish and 51.4% in British populations[31].

Since TAP genes are located within the MHC class II region and function in the processing of antigenic peptides, they are very good candidates for especially MHC-linked diseases and are also likely susceptibility/severity factors for other diseases. The polymorphisms of TAP genes affect the specificity of antigen binding and presentation. Therefore, they might have important roles in the course of some diseases. There are many studies on the association of TAP gene polymorphisms with different diseases.

It is known that CF patients are susceptible to lung infections with some specific agents. In a previous study Garred et al.[12] showed that there is an association between MBL variants and the severity of the lung disease and survival in CF. We thought that TAP 1 and TAP 2 genes might be other candidates having modifying effects on the CF phenotype. Our results indicate that TAP 1 gene polymorphism frequency at positions 333 and 637, and TAP 2 gene polymorphisms at position 665 are significantly different between CF patients and control subjects. There was an increased frequency of Val at position 333 and Gly at position 637 of TAP 1 and of Ala at position 665 of TAP 2 in CF patients.

Table IV. Distribution of TAP 1 and TAP 2 gene variants in three different populations

Position	Polish population* n: 128 %	British population* n: 290 %	Turkish population n: 100 %
TAP 1-333-Ile	98.7	97.9	96.0
TAP 1-333-Val	31.0	33.4	25.0
TAP 1-637-Asp	98.7	99.3	86.0
TAP 1-637-Gly	22.5	31.4	18.0
TAP 2-379-Ile	25.0	25.2	26.0
TAP 2-379-Val	97.5	99.7	75.0
TAP 2-565-Ala	100.0	99.7	99.0
TAP 2-565-Thr	23.1	19.0	5.0
TAP 2-665-Ala	46.9	51.4	1.0
TAP 2-665-Thr	96.2	95.5	100.0

n: number of subjects.
* Foley et al.[31]

In order to further analyze the role of TAP gene polymorphisms in the severity of the disease, we analyzed the ΔF508/ΔF508 CF patients (n: 36). From the comparison of this group with the control subjects, a significant difference in the overall genotype frequency of TAP 2 position 665 was observed. The frequency of Ala was also higher in this CF patient group when compared to control subjects. Thr/Ala and Ala/Ala genotypes again were overrepresented in ΔF508/ΔF508 CF patients.

This is the first study analyzing the association between TAP gene polymorphisms and CF phenotype. Furthermore, it is the first report of TAP gene polymorphism frequency in the Turkish population. We demonstrated that TAP genes in addition to other factors such as MBP variants might play a modifying role in the course of the CF phenotype. It will be interesting to see results from other populations to determine if TAP genes have a role in the modification of the CF disease phenotype.

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