

Allergens, IgE, mediators, inflammatory mechanisms

Polymorphism in transporter antigen peptides gene (TAP1) associated with atopy in Tunisians

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Background: Transporter antigen peptide 1 (TAP1) and TAP2 gene products form a transporter molecule involved in antigen presentation. Polymorphic residues have been described in both genes and could have functional consequences in the immune response.

Objective: We designed a case-control study to investigate the potential association of polymorphism of the TAP1 gene with atopy.

Methods: We used the amplification refractory mutation system polymerase chain reaction to characterize TAP1 gene polymorphism in 84 unrelated Tunisian patients with atopy and 81 healthy control subjects.

Results: Analysis of TAP1 polymorphism in Tunisian patients with atopy and in unaffected control subjects demonstrates a high relative risk (RR) of atopy in carriers of a codon (d) corresponding to a glycine at position 637 of the TAP1-B and TAP1-D alleles. The relative risk of allergic asthma is markedly higher in homozygotes (d/d) (RR = 22; $p \leq 0.0001$). The TAP1-D allele, not observed in European populations, has a frequency of 5% in the Tunisian control subject group. A major increase of the frequency (f) of the D allele is observed in patients with allergic asthma (f = 35%) and in those with allergic rhinitis (f = 22%), indicating a high relative risk of allergic asthma (RR = 10.2; $p \leq 0.0001$) and of allergic rhinitis (RR = 5.4; $p \leq 0.005$) in individuals carrying this allele. DD homozygotes were found only among patients with allergic asthma (23% of patients with asthma). Further evidence of the strong association between TAP1 polymorphism and atopy was provided by the finding that atopy is transmitted by inheritance of the glycine-637 marker.

Conclusions: Tunisian persons carrying the glycine-637 of the TAP1 protein may have an increased risk of atopy. Specific association was found between the homozygous TAP1 D/D genotype and allergic asthma. (*J Allergy Clin Immunol* 1997;99:216-23.)

Key words: TAP1 polymorphism, amplification refractory mutation system polymerase chain reaction, atopy, allergic asthma

Both environmental and genetic factors are involved in the onset of atopy. Although many studies are in agreement that the genetic component is a major determining factor of the disease, there is little information on the physical mapping and sequencing of the disease genes.¹ Defining atopy as a positive skin test response, and/or elevated total serum IgE, and/or elevated specific IgE, Cookson et al.²⁻⁵ reported that atopy was linked to a marker on chromosome 11q, by using an autosomal

Abbreviations used

ARMS-PCR:	Amplification refractory mutation system polymerase chain reaction
OR:	Odds ratio
RR:	Relative risk
TAP:	Transporter antigen peptides

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dominant model of inheritance. Although these results strongly suggest the presence of at least one major gene for atopy on chromosome 11q, several independent reports have shown nonlinkage of atopy to this locus.⁶⁻¹¹ More recently, Marsh et al.¹² and Meyers et al.¹³ described evidence of linkage of a locus for total serum IgE levels to chromosome 5 q. The overall agreement of these studies is the polygenic nature of atopy. In this regard, we have been interested in identifying a candidate gene predisposing to atopy.

Several reports indicate underlining immunoregulatory abnormalities in atopy, including increased IgE

TABLE 1. Oligonucleotide primers used for ARMS PCR typing of TAP1

Polymorphic site	Name	Oligonucleotide sequence	Specificity	Product size
TAP 1 position 333	A1	5'-CCCTGCACTGAGATTTCAGACCTCTGGAG-3'	5'-flanking sequence	Control; 533 bp
	A2	5'-GATCAGTGTCCCTCACCATGGTCACCCGGA-3'	TAP1, Ile-333	Ile-333; 241 bp
	A3	5'-GGGCAGAAGGAAAAGCAGAGGCAGGGTCAC-3'	TAP1, Val-333	Val-333; 352 bp
	A4	5'-ACCTGGGAACATGGACCACAGGACAGGGT-3'	3'-flanking sequence	
TAP1 position 637	A5	5'-CATCTTCCCAGAATCTCCCTATCCAGCTA-3'	5'-flanking sequence	Control; 429 bp
	A6	5'-CATCTTGGCCCTTTGCTCTGCAGAGGTACA-3'	TAP1, Asp-637	Asp-637; 307 bp
	A7	5'-ACCCCTGACAGCTGGCTCCAGCCTCCC-3'	TAP1, Gly-637	Gly-637; 180 bp
	A8	5'-TGGGGAGGCATCCAATGGAAGTGGATTGG-3'	3'-flanking sequence	

Four oligonucleotides are included in a single reaction mix. The expected sizes of the three amplified products (one constant, two variable) that can be produced in each reaction are listed in the final column.

production and decreased CD8⁺ suppressor/cytotoxic T cell numbers and function.^{14, 15} The HLA class II region contains two interferon- γ -inducible genes, for transporter antigen peptide 1 (TAP1) and TAP2. These genes are of particular interest because their involvement in immune response regulation makes them candidate atopy susceptibility genes.

TAP1 and TAP2 genes encode a heterodimeric molecule involved in endogenous antigen processing.¹⁶⁻¹⁸ TAP molecules are responsible for the translocation of intracellular antigenic peptides across the endoplasmic reticulum membrane where they bind to class I molecules. The class I antigen-antigenic peptide complex is then expressed on the cell surface and recognized by CD8⁺ T cells. Recent work¹⁹ has shown that exogenous antigens can be channeled into the endogenous pathway, presented by HLA class I molecules, and recognized by CD8⁺ T lymphocytes. Thus TAP gene products are thought to be among factors dictating the nature of endogenous and exogenous antigen stimuli.

Both TAP gene products are members of the adenosine triphosphate (ATP)-binding cassette superfamily, which includes proteins known to transport peptides across bacterial cell walls. TAP1 and TAP2 molecules possess one hydrophobic domain and one ATP-binding domain. Of the previously reported TAP1 polymorphism,²⁰ one (at position 333) is located within the hydrophobic domain and one (at position 637) within the ATP-binding domain. Variation at either position may alter the specificity of the substrate and the function of the TAP protein. Therefore the polymorphism of TAP genes could have functional consequences that may alter immune response regulation.

The aim of this study was to assess the degree of the TAP1 gene polymorphism in healthy subjects and in patients with atopy in Tunisia.

METHODS

Patients

The gene and allele frequencies of the TAP1 gene were determined in a group of 81 healthy subjects and 84 unrelated patients with atopy stratified into three groups: 48 patients with allergic asthma, 45 patients with allergic rhinitis, and 20 patients with atopic dermatitis. Of the 48 patients with asthma, 15 had allergic rhinitis. Fourteen patients had atopic dermatitis asso-

ciated with allergic rhinitis. The patients (34 girls and 50 boys) had a mean age of 10 ± 6 years. Patients were recruited from the departments of pediatrics, pneumology, and dermatology of hospitals in Monastir and Mahdia. The diagnosis of atopy was based on several criteria including clinical signs of atopic disease; a positive skin test response to one or more of a series of common allergens including *Dermatophagoides pteronyssinus*, cat allergen, grass mix, and tree pollen mix; a family history of atopy; elevated total IgE concentration in serum; presence of eosinophilia. The diagnosis of allergic asthma was made according to the American Thoracic Society criteria.

Control subjects (29 women and 52 men with a mean age of 24 ± 5 years) were unrelated healthy blood donors. A standard questionnaire was used to select subjects who had no evidence of any personal or family history of atopy. Control subjects and patients were selected from the same population living in the Monastir and Mahdia region (the middle coast of Tunisia). All subjects gave written informed consent.

DNA extraction

Genomic DNA was extracted from peripheral blood leukocytes by a salting-out procedure.²¹ In brief, 5 ml of blood was mixed with Triton lysis buffer (0.32 mol/L sucrose, 1% Triton X-100, 5 mmol/L MgCl₂, H₂O, 10 mmol/L Tris-HCl, pH 7.5). Leukocytes were spun down and washed with water. The pellet was incubated with proteinase K at 56°C and subsequently salted out at 4°C by use of a saturated NaCl solution. Precipitated proteins were removed by centrifugation. The DNA in the supernatant was precipitated with ethanol. The DNA pellet was dissolved in 400 μ l water.

Oligonucleotides

To analyze the single polymorphic site of TAP1 gene, four oligonucleotides were included in a single reaction mix. Nucleotide sequences of the primers are listed in Table I.

Typing TAP1 polymorphism by amplification refractory mutation system polymerase chain reaction (ARMS PCR)

Polymorphism within TAP1 has been characterized by Colonna et al.,²⁰ who identified two polymorphic residues, Ile for Val at position 333 and Asp for Gly at position 637. In this study we have used the ARMS PCR technique²² to characterize TAP1 polymorphism in Tunisian patients with atopy. This technique has advantages over other commonly used typing techniques. It does not require radioisotopes, digestion by restriction endonucleases, or sequence analysis. After PCR amplification, no further steps are required other than inspec-

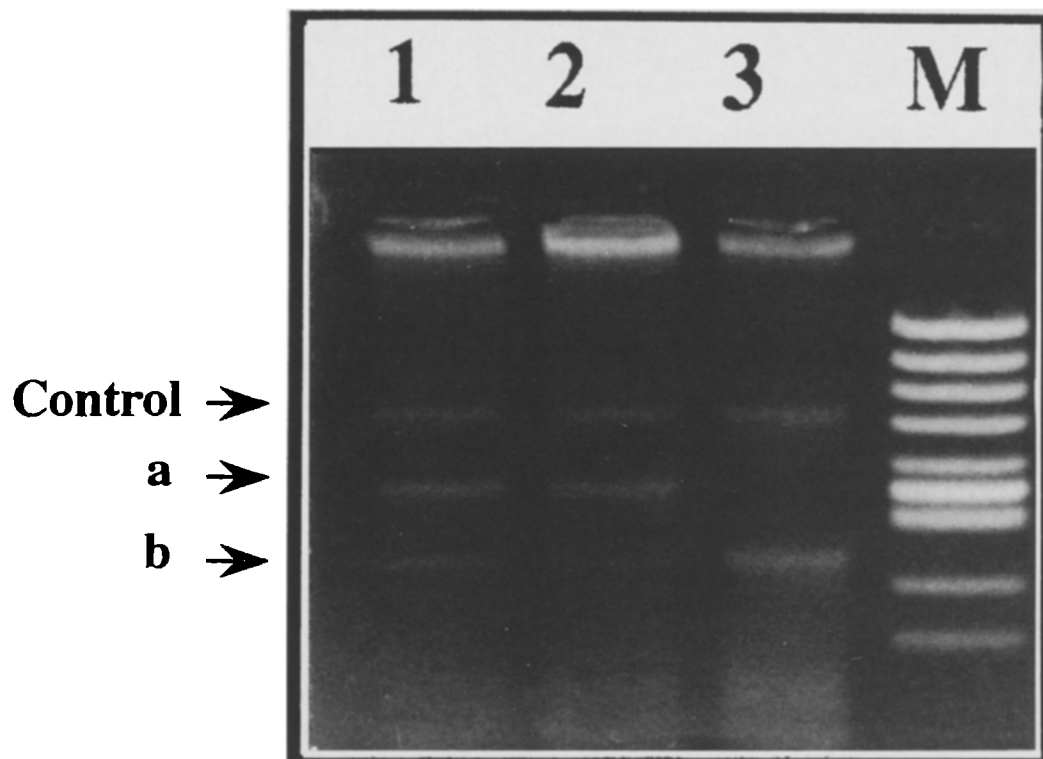


FIG. 1. Typing TAP1 position 333 by ARMS PCR. Four oligonucleotides were included in each PCR mix. Two of these, one sense (TAP1/A2) and one anti-sense (TAP1/A3), were designed such that their 3' terminal nucleotides were complementary to one of the two variants of TAP1 nucleotide 1207, which encodes amino acid 333. TAP1/A2 would only amplify efficiently if TAP1 nucleotide 1207 was A, encoding Ile-333. TAP1/A3 would only amplify efficiently if TAP1 nucleotide 1207 was G, encoding Val-333. The other two oligonucleotides, TAP1/A1 (sense) and TAP1/A4 (anti-sense), were complementary to flanking sequences located at asymmetrical distances on either side of nucleotide 1207. As illustrated, amplification resulted in a constant control product of 533 bp, together with a single product of 241 bp if the sample was homozygous for Ile-333, a single product of 352 bp if the sample was homozygous for Val-333, or both products if the sample was heterozygous. The products of three amplified reaction mixes after electrophoresis in 2% agarose are shown. *a*, 352 bp Val-333 specific product; *b*, 241 bp Ile-333-specific product. Lane *M*, Molecular weight markers; lanes 1 and 2, heterozygotes; lane 3, Ile-333 homozygote.

tion after agarose gel electrophoresis. In this method a product amplified by the two-allele-nonspecific flanking oligonucleotides is used as an internal control. Because the control product is amplified by oligonucleotides that are also used to amplify the allele-specific product, loss of amplification by either flanking oligonucleotide (a potential false-negative result) will also result in loss of the control product. Genomic DNA samples (1 μ g) were amplified in 100 μ l of reaction mixtures containing 0.25 μ g of each oligonucleotide primer (four primers), 200 μ mol/L dNTPs, 1 $\times$ Taq DNA polymerase buffer, and 2 units of Taq DNA polymerase (Amersham, France), overlaid with mineral oil. Reaction conditions used with the thermal cycler (Thermolyne) were as follows: 95° C for 5 minutes; 35 cycles of 94° C for 1 minute, 58° C for 2 minutes, 72° C for 2 minutes; 72° C for 10 minutes. Reaction products were separated on a 2% agarose gel and stained with ethidium bromide.

An ethidium bromide-stained gel is shown in Fig. 1 to illustrate a typical TAP1 polymorphism analysis.

Statistical analyses

The chi square test with Yates' correction was used to test for significant association between disease (allergic asthma, allergic

rinitis, or atopic dermatitis vs controls) and TAP1 alleles or genotypes. Relative risk associated with a particular allele (or codon or genotype) was estimated by the odds ratio (OR) formula²³: $OR = n_1 \cdot n_4 / n_2 \cdot n_3$, where n_1 is the proportion of patients carrying the allele, n_2 is the proportion of control subjects with the allele, and n_3 and n_4 are the corresponding proportions of individuals in patient and control groups not carrying the allele. The OR was tested by using a chi square distribution, and the null hypothesis being tested was $OR = 1$.²⁴ Statistics were performed by use of StatView software, version 4.0 (Abacus Concepts, Berkeley, Calif.).

RESULTS

Frequency of TAP1 alleles in the Tunisian population

The possible alleles of TAP1, based on known polymorphism, are shown in Fig. 2 together with the frequency at which each allele was observed in Tunisian (control subjects of this study) and in European populations.²⁵

The TAP1 allele frequencies in the Tunisian group

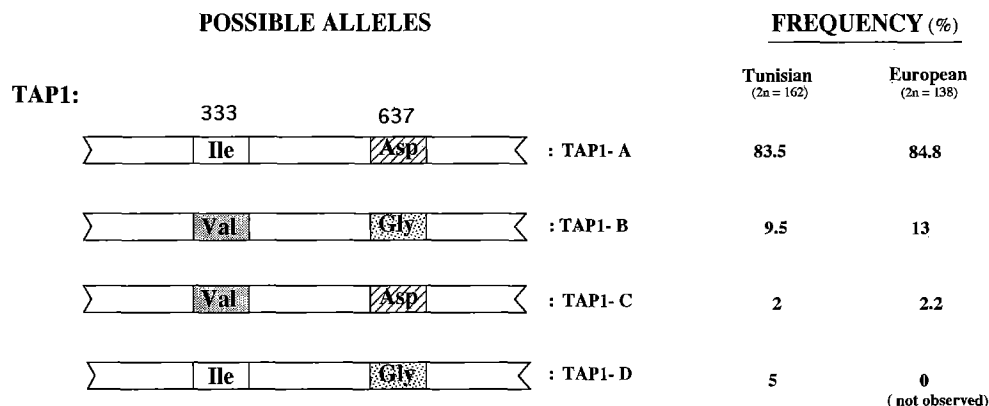


FIG. 2. Alleles of TAP1. The four possible TAP1 alleles are illustrated. The frequency at which each allele was observed in Tunisian and European²⁴ populations is shown as a proportion of the total number of chromosomes analyzed.

TABLE II. TAP1 gene and allele frequencies (%) in control subjects and atopic patients stratified in three groups

Codon			Control group (2n = 162)	AA group (2n = 96)		AR group (2n = 90)		AD group (2n = 40)	
333	637		%	%	p value	%	p value	%	p value
a	Val		11	20	NS	22	NS	25	0.01
b	Ile		89	80		78		75	
c		Asp	86	46		55		62	
d		Gly	14	54	0.0001	45	0.0001	32	0.005
A	Ile	Asp	84	45	0.0001	55	0.0001	62	0.0008
B	Val	Gly	9	19	NS	22	0.019	25	0.005
C	Val	Asp	2	1	NS	1	NS	0	NS
D	Ile	Gly	5	35	0.0001	22	0.001	13	NS

The chi square test with Yates' correction was used to determine whether significant differences (*p* values) in gene and allele frequencies were observed when each patient group was compared with control subjects. The values represent the percentage of the total number of chromosomes; *n* = number of individuals in each group; 2*n* = number of chromosomes scored to determine gene and allele frequencies. a, b, c, and d denote the gene (codon). A, B, C, and D denote the allele.

AA, Allergic asthma; AR, allergic rhinitis; AD, atopic dermatitis; NS, not significant.

provide a contrast to those found in European populations. A most striking difference is the finding that the TAP1-D allele, which was not observed in Europeans, was represented in the Tunisian population. A decrease of the TAP1-B allele was also observed in Tunisians compared with Europeans.

Association between allergic asthma and TAP1 polymorphism

Table II shows gene and allele frequencies for TAP1 in the three patient groups (allergic asthma, allergic rhinitis, and atopic dermatitis) and the control group. The distribution of TAP1 genotypes among control and patient groups is shown in Table III.

A major decrease of the A/A genotype was observed in the group with asthma compared with the control population (38% vs 73%; *p* ≤ 0.0001), resulting in a significantly reduced frequency of the TAP1-A allele in patients with asthma (45% vs 84% in control subjects; *p* ≤ 0.0001).

A high relative risk (RR) of allergic asthma (RR = 7.2; *p* ≤ 0.0001) was associated with the Gly-637 codon (d) of the TAP1-B and TAP1-D alleles. The RR of asthma was even higher in homozygotes (d/d) (RR = 22; *p* ≤ 0.0001). The increased gene frequency of TAP1 Gly-637 resulted in increased frequencies of the TAP1-B and TAP1-D alleles in the group with asthma, but only the frequency of the D allele reached statistical significance (35% vs 5% in control group; *p* ≤ 0.0001). This result indicates a high RR for allergic asthma in carriers of the TAP1-D allele (RR = 10.2). It is of interest that homozygous D/D carriers were found only in the group with allergic asthma (23% of the patients with asthma, including three patients with both allergic asthma and allergic rhinitis).

Association between allergic rhinitis and TAP1 polymorphism

As in patients with allergic asthma, a highly significant decrease of the A/A genotype was observed in patients with

TABLE III. Distribution (%) of TAP1 genotypes in control subjects and in the three patient groups

Genotype	Control group (n = 81)	AA group (n = 48)	p value	AR group (n = 45)	p value	AD group (n = 20)	p value
	%	%		%		%	
a/a	2	4	NS	0	NS	10	NS
a/b	18	31	NS	46	0.0001	30	NS
d/d	4	48	0.0001	24	0.0002	15	0.01
c/d	20	12	NS	33	NS	45	0.0003
A/A	73	38	0.0001	35	0.0001	40	0.0001
A/B*	12	8	NS	24	NS	25	0.03
A/C	2	2	NS	2	NS	0	NS
A/D	6	4	NS	11	NS	20	0.006
B/B	0	4	NS	0	NS	10	0.004
B/C	2	0	NS	0	NS	0	NS
B/D	4	21	0.0006	20	0.002	5	NS
C/C	0	0	NS	0	NS	0	NS
C/D	0	0	NS	0	NS	0	NS
D/D	0	23	0.0001	6†		0	NS

Each value represents the percentage of the number of individuals in each group. The chi square test with Yates' correction was used to determine significance of differences observed between patients and control subjects.

AA, Allergic asthma; AR, allergic rhinitis; AD, atopic dermatitis; NS, not significant.

*When an individual carried heterozygosities for both TAP1 codon 333 and TAP1 codon 637, two genotypes, A/B or C/D, were possible. However, the C/D genotype was estimated to be very rare, and such individuals were considered to be of the A/B genotype as proposed by Powis et al.²²

†Corresponds to three patients with allergic asthma associated with allergic rhinitis.

allergic rhinitis compared with the control population (35% vs 73%, $p \leq 0.0001$). Consequently, the TAP1-A allele was found at lower frequency in the group with allergic rhinitis than in control subjects (55% vs 84%, $p \leq 0.0001$).

The gene frequency of the TAP1 Gly-637 was significantly higher in patients with allergic rhinitis (45% vs 14% in control subjects, $p \leq 0.0001$), which indicates a high RR of allergic rhinitis (RR = 5) in individuals carrying the Gly-637 codon. Allergic rhinitis was also found to be associated with the TAP1-D allele (RR = 5.4; $p \leq 0.0001$), with a particularly high frequency of B/D heterozygotes compared with control subjects ($p < 0.005$). In contrast to the group with allergic asthma, the TAP1-B allele occurred at a significantly higher frequency in the group with allergic rhinitis than in the control group (22% vs 9%, $p \leq 0.02$).

Association between atopic dermatitis and TAP1 polymorphism

As in allergic asthma and rhinitis, the TAP1-A allele and the frequency of the A/A homozygous genotype were found to be decreased in patients with atopic dermatitis compared with control subjects. The frequencies of TAP1 Val-333 and Gly-637 codons were significantly higher in patients with atopic dermatitis than in control subjects, resulting in an increased frequency of TAP1-B allele in this group of patients.

The TAP1 B/B and A/D genotypes were present at significantly higher frequencies in patients with atopic dermatitis compared with control subjects (10% vs 0% for the B/B genotype and 20% vs 6% for the A/D genotype). This result, along with the finding that no

significant association was seen between the TAP1-D allele and atopic dermatitis, distinguishes susceptibility to atopic dermatitis from that to allergic asthma and rhinitis.

TAP1 polymorphism in families of Tunisian atopic patients

To determine whether atopy was associated with inheritance of the Gly-637 (d) of the TAP1-B and TAP1-D alleles, we examined the family members of four atopic, d-carrying probands, for the presence of this marker. The results of these analyses are shown in Fig. 3. The transmission of atopy in family A is associated with inheritance of TAP1 Gly-637 (d) of the B allele. The proband with atopic asthma (subject 4) was a heterozygous (d^+/d^-) child who had received the TAP1 Gly-637 from her mother who had atopic asthma. All members of this family who had atopic asthma were carriers of the TAP1 Gly-637. The unaffected members (the father and one of the sons) were negative for this marker. In this family the TAP1 Gly-637 of the B allele shows a dominant mode of transmission of atopy.

In families B, C, and D, the transmission of atopy is associated with inheritance of TAP1 Gly-637 of the D allele. In family B, the proband (subject 6) and his brother were homozygotes (d^+/d^+) with allergic asthma. Their heterozygous parents were not affected. Both the grandmother and her homozygous (d^+/d^+) daughter (subjects 2 and 3) had atopic asthma. In family C, the proband (subject 3) and all other members with atopic asthma were homozygotes (d^+/d^+). The unaffected members were heterozygotes (d^+/d^-). In family D, the proband (subject 3) and her father were homozygotes

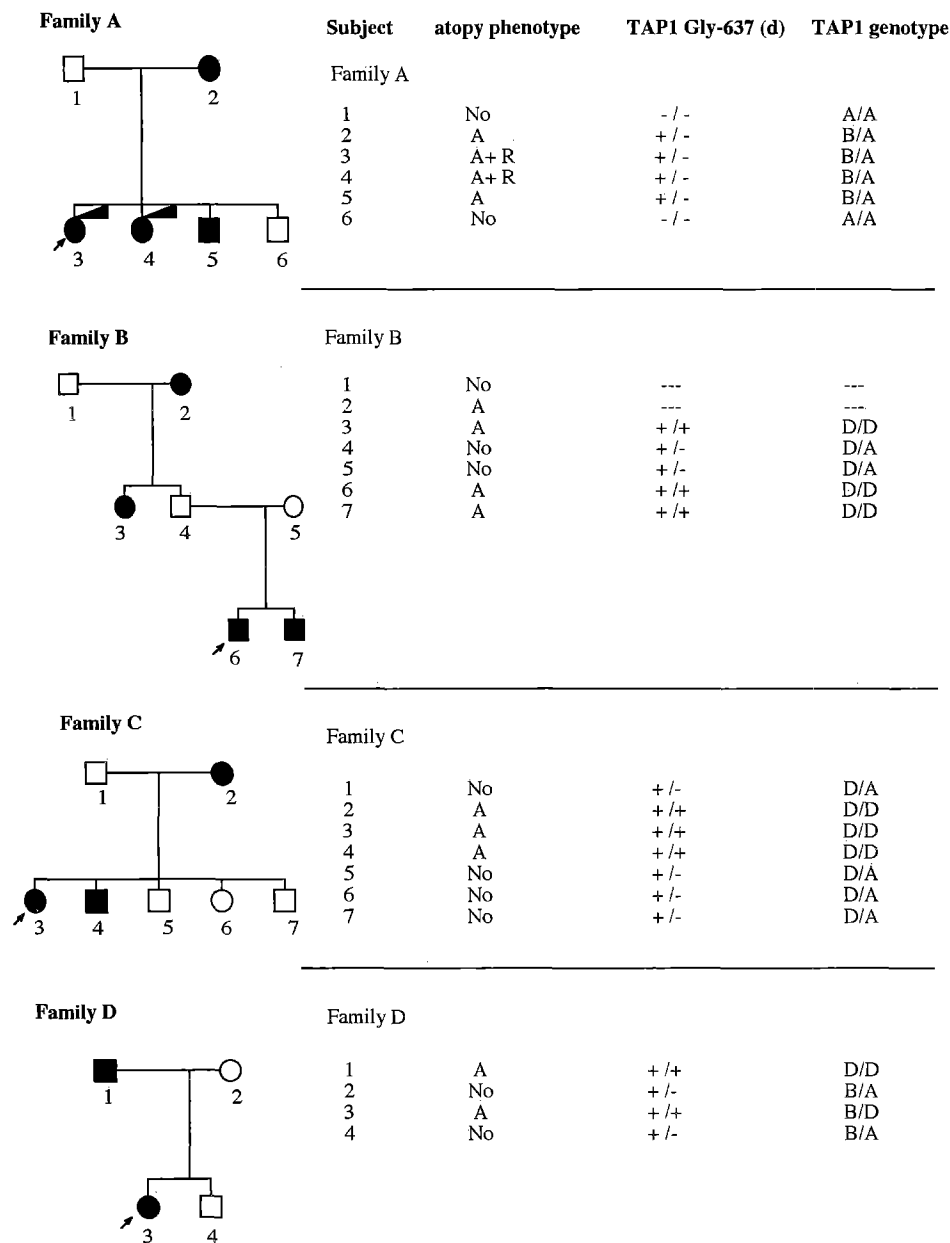


FIG. 3. Pedigrees and clinical features of families of four Tunisian patients with atopy. *Solid circles and squares* represent female and male family members with atopy respectively. *A*, Allergic asthma; *R*, rhinitis; *arrows*, probands. In family A the proband and her sister are monozygotic twins. Genotypes for TAP1 Gly-637 are as follows: +/+, Gly-637 homozygote; +/-, Gly-637 heterozygote; and -/-, Gly-637 negative in both chromosomes.

(d⁺/d⁺) with allergic asthma. The proband's mother and brother were unaffected heterozygotes (d⁺/d⁻). In families B, C, and D, the inheritance of TAP1 Gly-637 shows a recessive mode of disease transmission.

DISCUSSION

Several reports have indicated that different HLA products and genes may be risk factors for or protective factors against the development of atopy.²⁶⁻³² However, no association with specific alleles has been found.³⁰⁻³² In this study we report a strong association between

HLA-linked TAP1 gene polymorphism and atopy in the Tunisian population.

First, we showed that TAP1 allele distributions in Tunisians are different from those in European whites. The most striking difference is that the D allele, which was not observed in Europeans, was represented in Tunisians. This finding contrasts with that reported for the HLA class I and class II genes, in which the greatest similarities of gene frequencies were seen between Tunisians and Europeans.³³

Comparison of TAP1 gene and allele frequencies in

atopic patients and control subjects indicated a marked decrease of the TAP1 A/A homozygous genotype in all three groups with atopy, resulting in a decrease of the TAP1-A allele in these groups. This result suggests that the TAP1 A/A genotype may be considered to be a protective marker against atopy.

A high RR of atopy was associated with the Gly-637 codon (d) of the TAP1-B and TAP1-D alleles. The RR of allergic asthma was even higher in homozygotes (d/d). The increased gene frequency of TAP1-Gly 637 in atopic patients led to an increase of the rare TAP1-D allele in all three groups with atopy. However, the frequency of this allele did not reach statistical significance in the atopic dermatitis group. Whether this is a result of the relatively low number of patients with eczema or of a specificity of atopic dermatitis cannot be stated with certainty.

The homozygous D/D genotype was found exclusively in patients with allergic asthma. More than 20% of patients with allergic asthma were found to carry the homozygous D/D genotype. This result suggests that the homozygous TAP1 D/D genotype may be a specific risk factor for allergic asthma. Because no prognostic factors are available to predict whether a child with atopic dermatitis will have asthma, allergic dermal respiratory syndrome³⁴ remains a major concern in dermatology and pediatrics clinics. The TAP1 D/D genotype seems to be an attractive candidate for a prognostic factor for the dermal respiratory syndrome in Tunisians.

Further support for the strong association between TAP1 Gly-637 and atopy was provided by the finding that atopy is associated with inheritance of this marker in families of atopic patients. Both dominant and recessive modes of disease transmission were found to be associated with inheritance of the TAP1 Gly-637.

Because total serum IgE can be affected by several factors including allergen exposure, parasitic infection, age, sex, and smoking status, we used a definition of atopy that was based on a combination of several clinical criteria rather than on total serum IgE concentration. Consequently, the strong association found between TAP1 polymorphism and atopy cannot be assigned to a specific aspect of the disease. However, examination of TAP1 polymorphism in atopic patients with regard to total serum IgE showed no association with any TAP1 alleles (data not shown), suggesting that the TAP1 gene may not be involved in the control of total IgE production.

Although previous analyses of TAP polymorphism have revealed limited linkage disequilibrium between TAP and HLA class II genes,³⁵ it will be of interest to determine whether linkage disequilibrium occurs between TAP1 alleles and those of TAP2 or HLA class II genes in the Tunisian population.

Although this study, showing the strong association of TAP1 alleles with atopy in Tunisians, suggests that the TAP molecules play a role in susceptibility to the different phenotypic expressions of atopy, further studies are needed to clarify their role in the pathogenesis of the disease. Our data also suggest a need for large-scale

TAP1 typing in Tunisians with asthma and atopy. Replication of our findings in other populations will be of use in determining whether the relations between these risk factors and the development of allergic asthma and atopy can be generalized.

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