CONCISE REPORT

TAP1 and TAP2 polymorphisms analysis in northwestern Colombian patients with systemic lupus erythematosus

P A Correa, J F Molina, L F Pinto, M Arcos-Burgos, M Herrera, J-M Anaya

Ann Rheum Dis 2003;**62**:363–365

Objective: To determine the influence of TAP1 and TAP2 alleles in northwestern Colombian patients with systemic lupus erythematosus (SLE).
Methods: Unselected patients with SLE (n=140) and controls (n=120) matched for sex, age, and ethnicity were analysed. Clinical manifestations, clinical activity, and severity of disease were recorded. Autoantibodies were detected by enzyme linked immunosorbent assay (ELISA). TAP1 and TAP2 polymorphisms were determined by amplification refractory mutation system-polymerase chain reaction. A Hardy-Weinberg equilibrium test, microdifferentiation analysis, linkage disequilibrium analysis, and haplotype and allele frequency comparisons were performed.
Results: The TAP2 variant Val379/Ala565/Ala665 (allele TAP2*0201) was associated with SLE (56% *v* 39%; odds ratio=2, 95% confidence interval 1.22 to 3.30, p_c=0.03). There was no stratification between patient and control samples. Linkage disequilibrium between TAP1 and TAP2 loci was found in controls but not in patients. An excess in the number of heterozygotes in the TAP2 locus was found in patients. No association between TAP1 and TAP2 variants and the presence of autoantibodies, clinical expression, or severity of disease was found.
Conclusions: The TAP2 locus influences susceptibility to SLE in our patient group; however, it has no significant effect on the immune response or on the clinical course of the disease.

Systemic lupus erythematosus (SLE) is a clinically heterogeneous autoimmune disease of unknown aetiology, in which environmental, genetic, and ethnic factors may have an important role. The human leucocyte antigen (HLA) HLA-DR2 and DR3 alleles have been associated with SLE in numerous studies, mainly with the production of anti-Ro and anti-La autoantibodies.[1] Other susceptibility candidate genes may include tumour necrosis factor α, mannose binding protein, interleukin 10, angiotensin converting enzyme, and deficiencies of components of the complement system.[1 2]

Transporters associated with antigen processing (TAP) genes (TAP1 and TAP2) may also be considered as a susceptibility factor for SLE. TAP1 and TAP2 are polymorphic genes located in the class II region of the major histocompatibility complex (MHC) between HLA-DP and HLA-DQ genes. TAP genes code for a homologous molecule to the ATP binding cassette (ABC transporters) that conforms to the chaperons ABC superfamily.[3] TAP is a dimer responsible for transportation of antigenic peptides bound to HLA class I, but the contribution of TAP molecules to endogenous processing of peptides bound to HLA class II has also been reported.[4] Four potential alleles for the TAP1 gene and eight alleles for the TAP2 gene have been reported with some single nucleotide polymorphism in each gene.[5] TAP genes are interesting candidates for the study of genetic susceptibility in autoimmune diseases given their location at the MHC class II region, their functional relation with HLA molecules, and their polymorphism.

A few studies have evaluated the association of TAP1 and TAP2 with SLE.[6–10] In the Latin-American population no information exists about these genes and SLE. Thus, we examined the influence of TAP1 and TAP2 polymorphisms on the susceptibility, clinical expression, and severity of SLE, as well as on the production of autoantibodies in northwestern Colombian patients.

PATIENTS AND METHODS

Study group

This was a multicentre, cross sectional study. The patients with SLE were recruited in Medellin, Colombia. All patients fulfilled four or more of the American College of Rheumatology (ACR) criteria for the classification of SLE.[11] Healthy people unrelated to the patients, without inflammatory or autoimmune disease, matched to patients by age (±5 years), sex, and ethnicity were included as controls. This study had the approval of the local ethics committee.

Clinical and laboratory features

The clinical and laboratory variables associated with SLE, including each feature of the revised ACR criteria, were evaluated.[11] These clinical manifestations were defined as follows: (*a*) arthritis: non-erosive arthritis affecting two or more peripheral joints, characterised by tenderness, swelling, or effusion; (*b*) malar rash; (*c*) photosensitivity; (*d*) alopecia; (*e*) discoid lupus; (*f*) Raynaud's phenomenon; (*g*) renal disease, as evidenced by a renal biopsy result demonstrating World Health Organisation (WHO) class II–V histopathology, active urinary sediment, or proteinuria >500 mg/24 h; (*h*) neurological disease, as evidenced by seizures without any other definable cause, or psychosis lacking any other definable cause, or other conditions such as peripheral neuropathy, stroke, transverse myelitis, chorea, or other central nervous system lesions directly attributable to SLE in the absence of other causes; (*i*) pleuritis: pleural rub and/or effusion and/or typical pleuritic pain; (*j*) pericarditis: documented by electrocardiogram, rub, or evidence of pericardial effusion; (*k*) autoimmune haemolytic anaemia, with a packed cell volume of <0.35, reticulocyte count >4%, and positive Coombs test; (*l*) leucopenia, white cells $<4.0\times10^9$/l; (*m*) thrombocytopenia, platelets $<100\times10^9$/l; (*n*) arterial or venous thrombosis diagnosed on clinical grounds and confirmed by complementary tests.

The severity of the disease and the organic damage, was evaluated using the systemic lupus international collaborating clinics (SLICC) damage index (SDI).[12]

Abbreviations: ACR, American College of Rheumatology; CI, confidence interval; MHC, major histocompatibility complex; OR, odds ratio; SLE, systemic lupus erythematosus

Table 1 Clinical and serological characteristic of 140 patients with SLE

Characteristics	
Age (years), mean (SD) [range]	31 (1.3) [13–64]
Age of onset (years), mean (SD) [range]	25 (1.1) [9–61]
Duration of SLE (years), mean (SD) [range]	6.3 (0.7) [1–30]
Cutaneous involvement (%)	73
Musculoskeletal involvement (%)	100
Raynaud's phenomenon (%)	50
Serositis (%)	22
Nephritis (%)	47
Neurological involvement (%)	20
Thrombosis (%)	10
Cytopenia (%)	81
Hypocomplementaemia (%)	82
SLICC/ACR, mean (SD) [range]	1.3 (0.2) [0–8]
Anti-DNA antibodies* (%)	71
Anti-Ro antibodies* (%)	35
Anti-La antibodies* (%)	20
Anti-Sm antibodies* (%)	33
Anti-RNP antibodies* (%)	48

*By ELISA, using commercial kits.

Table 2 Frequencies of TAP1 and TAP2 polymorphisms in 140 patients with SLE and 120 controls

Variants TAP1	Positions 333/637	Patients with SLE No (%)	Controls No (%)
*0101/02	Ile, Asp	110 (79)	90 (75)
*02011/12	Val, Gly	11 (8)	5 (4)
*0301	Val, Asp	28 (20)	15 (13)
*0401	Ile, Gly	12 (9)	17 (14)
Variants TAP2	**Positions 379/565/665**		
*0101	Val, Ala, Thr	121 (86)	86 (72)
*0201	Val, Ala, Ala	79 (56)	47 (39)†
2C	Ile, Ala, Thr	13 (9)	25 (21)‡
2D	Ile, Thr, Thr	2 (1)	4 (3)
*0102	Val, Thr, Thr	4 (3)	5 (4)
*0103	Ile, Thr, Ala	0	0
2G	Val, Thr, Ala	0	2 (1)
2H	Ile, Ala, Ala	2 (1)	2 (1)

†OR=2, 95% CI 1.22 to 3.30, p=0.005, p_c=0.035; ‡OR=0.4, 95% CI 0.19 to 0.8, p=0.008, p_c=0.05.

TAP1 and TAP2 genotyping

TAP1 and TAP2 polymorphisms were identified by amplification refractory mutation system-polymerase chain reaction, using primers and conditions described elsewhere.[13] Each of the alternatives was typical for the diallelic sites corresponding to amino acid positions 333 and 637 in TAP1 and positions 379, 565, and 665 in TAP2.

Statistical and genetic data analysis

We examined the possibility of subdivision among patients and controls, the Hardy-Weinberg equilibrium, and linkage disequilibrium among loci as described elsewhere.[13] Differences between the patient and control marker gene frequencies were established by χ^2 test. Crude odds ratios (OR) were estimated and reported with its corresponding 95% confidence interval (CI). The Bonferroni correction for multiple comparisons was applied. A level of 5% was used to define statistical significance.

RESULTS

One hundred and forty patients with SLE and 120 controls were included in the study. Table 1 shows the clinical and serological characteristics of patients. In patients and controls the combination Ile333/Asp637 in TAP1 and Val379/Ala565/Thr665 in TAP2 were the most frequently found (table 2).

We found a significant difference in the frequencies of the single nucleotide polymorphisms among patients and controls for TAP2, but not for TAP1. The variant Val379/Ala565/Ala665 in TAP2 was found more often in patients than in controls (56% *v* 39%, OR=2, 95% CI 1.22 to 3.30, p_c=0.035). There were nine control homozygotes for TAP2 Ile379/Ala565/Thr665, whereas none of the patients was homozygotic for this combination (0% *v* 7.5%, OR=21.2, 95% CI 1.2 to 372, p=0.002). However, no effect of gene dose on susceptibility to disease was observed.

The haplotype frequencies among the TAP1-TAP2 interloci combination were estimated. The most common haplotypes in patients and controls were TAP1 Ile333/Asp637-TAP2 Val379/Ala565/Thr665 (39% and 36% respectively). Associations between TAP1 and TAP2 variants and the presence of autoantibodies, clinical parameters, or the severity of disease were not observed.

There was no stratification among patients and controls because the stratification test showed that the θ parameter was not significantly different from 0. TAP1 and TAP2 loci had a strong deviation from the Hardy-Weinberg expected proportion both in patients and controls. This deviation in the TAP1 locus was a consequence of a significant deficit in the number of heterozygotes. In the TAP2 locus, differences were found between patients and controls, with the former having an excess and the latter a deficit in the number of heterozygotes. Linkage disequilibrium between TAP1 and TAP2 loci was seen in controls (p=0.03) but not in patients (p=0.4).

DISCUSSION

Our results provided genetic evidence indicating that the TAP2 locus influences the susceptibility to SLE in our population. Firstly, an allelic misbalance in TAP2 was clearly seen between patients and controls. The TAP2 Val379/Ala565/Ala665 variant was associated with SLE. It is noteworthy that stratification among patients and controls was not found. Secondly, there was greater genetic selection in the TAP2 locus in patients because there was an excess in the number of heterozygotes. Thirdly, no linkage disequilibrium was found between TAP1 and TAP2 loci in patients, suggesting a high mutation rate at the TAP2 locus in this group. The analysis also disclosed that TAP variants have no significant influence on the immune response or the course of the disease.

The frequencies of TAP1 and TAP2 variants found in controls were similar to those observed in white, Brazilian, and African populations.[14] This result suggests that from an evolutionary point of view TAP genes are relatively ancient. TAP molecules have an essential role in antigen processing and presentation to cytotoxic T lymphocytes.[4] The TAP heterodimer is composed of TAP1 and TAP2 subunits which may function together or independently as homodimers, transporting antigenic peptides from the cytoplasm into the lumen of the endoplasmic reticulum.[15] The association of the TAP2 molecule with the selected peptide may depend on specific positions inside such peptide(s), which will link to positions 2, 3, 6, and 7 as well as the C-terminal extreme in TAP2.[16] Thus the role of TAP2 in SLE may correspond to a particular selection of peptides (autoantigens).

An association between the TAP2*01 allele and SLE and the synthesis of anti-Ro antibodies has been observed in Spanish subjects.[10] Nevertheless, in other ethnic groups, such as in white, oriental, African-American, and Caribbean subjects, no association was found.[6–9] The discrepancy among the studies might be due to ethnic diversity, as well as to different techniques used to examine TAP polymorphism.

In the patients we found no linkage disequilibrium between TAP1 and TAP2. However, a further study on HLA polymorphism should elucidate whether or not TAP alleles are in linkage disequilibrium with HLA in our population.

In summary, the TAP2 locus influences the susceptibility to SLE in a northwestern Colombian population; however, it has no effect on the immune response or on the clinical expression of the disease.

ACKNOWLEDGEMENTS

This study was supported by COLCIENCIAS (2213-04-1022-98), Bogotá, and the Corporación para Investigaciones Biológicas, Medellin, Colombia. We are grateful to Martha E Alarcon-Riquelme for her valuable advice on this study, and to James Tang for his fruitful comments on the manuscript.

Authors' affiliations
P A Correa, M Herrera, J-M Anaya, Corporación para Investigaciones Biológicas, Medellin, Colombia
J F Molina, Hospital Pablo Tobón Uribe, Medellin, Colombia
L F Pinto, J-M Anaya, Clínica Universitaria Bolivariana, Universidad Pontificia Bolivariana, Medellin, Colombia
M Arcos-Burgos, Universidad de Antioquia, Medellin, Colombia

Correspondence to: Dr J-M Anaya, Corporación para Investigaciones Biológicas, Cra 72A No 78B-141, Medellin, Colombia; janaya@cib.org.co

Accepted 12 August 2002

REFERENCES

1 **Harley JB**, Sestak AS, Willis LG, Fu SM, Hansen JA, Reichlin M. Genetic studies of Ro(SA-A) and La(SS-B) autoantibodies in families with systemic lupus erythematosus and primary Sjögren's syndrome. Arthritis Rheum 1989;32:413–19.
2 **Grossman JM**, Tsao BP. Genetic and systemic lupus erythematosus. Curr Rheumatol Rep 2000;2:13–18.
3 **Trowsdale J**, Hanson I, Mockridge I, Beck S, Townsend A, Kelly A. Sequences encoded in the clase II region of the MHC elated 'ABC' superfamily of transporters. Nature 1990;348:741–4.
4 **Momburg F**, Hämmerling GJ, Neefjes JJ. Tap peptide transporters and antigen presentation. In: Urban, RG and Chicz RM, eds. *MHC molecules: expression, assembly and function.* New York, NY: Chapman and Hall, 1996:36–63.
5 **Badmer JG**, Marsh K, Nakata K. Nomenclature for factors of the HLA system, 1998. Tissue Antigens 1999;53:407–46.
6 **Davies EJ**, Donn RP, Hillarby MC, Grennan DM, Ollier WE. Polymorphisms of the TAP2 transporter gene in systemic lupus erythematosus. Ann Rheum Dis 1994;53:61–3.
7 **Savage DA**, Ng SC, Howe HS, Ngai JL, Darke C, Hui KM. HLA and TAP associations in Chinese systemic lupus erythematosus patients. Tissue Antigens 1995;46:213–16.
8 **Takeuchi F**, Nakano H, Hong GH, Kuwata S, Ito K. Polymorphism or the TAP1 and TAP2 transporters genes in Japanese SLE. Ann Rheum Dis 1996;55:924–6.
9 **Ocal L**, Russell K, Beynon H, Cruickshank K, Lanchbury JS, Walport M, *et al.* Genetic analysis of TAP2 in systemic lupus erythematosus patients from two ethnic groups. Br J Rheumatol 1996;35:529–33.
10 **Martin-Villa JM**, Martinez-Laso J, Moreno-Pelayo MA, Castro-Panete, Martinez-Quiles N, Alvarez M, *et al.* Differential contribution of HLA-DR, DQ and TAP2 alleles to systemic lupus erythematosus susceptibility in Spanish patients: role of TAP2* alleles in Ro autoantibody production. Ann Rheum Dis 1998;57:214–19.
11 **Tan EM**, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–7.
12 **Gladman DD**, Urowitz MB, Goldsmith CH, Fortin P, Ginzler E, Gordon C, *et al.* The reliability of the systemic lupus international collaborating clinics/American College of Rheumatology damage index in patients with systemic lupus erythematosus. Arthritis Rheum 1997;40:809–13.
13 **Anaya JM**, Correa PA, Mantilla RD, Arcos-Burgos M. TAP, HLA-DQB1 and HLA-DRB1 polymorphisms in Colombian patients with primary Sjögren's syndrome. Semin Arthritis Rheum 2002;31:358–67.
14 **Tang J**, Freedman D, Allen S, Karita E, Musonda R, Braga C, *et al.* Genotyping TAP2 variants in North American Caucasians, Brazilians, and Africans. Genes Immun 2001;2:32–40.
15 **Antoniou A**, Ford S, Pilley E, Blake N, Powis S. Interactions formed by individually expressed TAP1 and TAP2 polypeptide subunits. Immunology 2002;106:182–9.
16 **Karttunen JT**, Trowsdale J, Lehner PJ. Antigen presentation: TAP dances with ATP. Curr Biol 1999;9:R820–4.