TAP polymorphism in patients with Behçet's disease

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Abstract

Objective—To determine if susceptibility to Behçet's disease (BD) is associated with polymorphism of HLA-DRB1, HLA-DQB1, and TAP1 and TAP2 genes.

Methods—Fifty eight Spanish BD patients and 116 ethnically matched unrelated healthy subjects were typed at the HLA-DRB1 and HLA-DQB1 loci using polymerase chain reaction/sequence specific oligotyping (PCR/SSO). TAP1 and TAP2 alleles were assigned using amplification refractory mutation system-PCR.

Results—TAP1C was absent in BD patients, but was found in 12.1% of control subjects ($p_{\text{corr}} < 0.05$; relative risk = 0.06). Additionally, a linkage disequilibrium between HLA-DQB1*0501 and TAP2B was observed in BD patients ($\Delta = 0.095$, $p_{\text{corr}} < 0.02$), but not in the control group ($\Delta = -0.0031$, $p > 0.05$).

Conclusions—The complete absence of TAP1C alleles in BD patients may indicate that TAP1 polymorphism is not without some significance in the development of BD. Furthermore, the existence of a linkage disequilibrium between HLA-DQB1*0501 and TAP2B in our patients suggests that the gene conferring susceptibility for BD is inherited as an extended haplotype in the population studied.

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A large number of immunogenetic studies in normal and patient populations have demonstrated that genes within the major histocompatibility complex (MHC), or closely linked to it, play a major role in the development of many human diseases. Behçet's disease (BD) is a multifaceted syndrome of unknown origin, characterised by oral aphthae, ocular lesions, cutaneous vasculitis, and genital ulcerations as major symptoms, and by arthritis, meningo-encephalitis, vascular, and visceral involvement as minor symptoms. Although the aetiology and pathogenesis of BD remain totally unknown, BD is associated with specific HLA alleles, the HLA-B51 antigen being the genetic marker most strongly associated with the disease in various ethnic groups.^{1,2} HLA-B51 is also a well defined marker of the ocular form of BD.^{1,2} However, this association is not absolute, raising the possibility that it could result from linkage disequilibrium with other loci. It is thus of interest to investigate the

involvement of other genes located in the human MHC, in BD susceptibility.

Stable cell surface presentation of peptides by HLA class I molecules requires active transport of antigenic peptides across the endoplasmic reticulum (ER) by products of two genes, TAP1 and TAP2 (transporters associated with antigen processing), which map in the MHC class II region,³ and are members of the ABC (ATP binding cassette) transporter superfamily. Several coding variants of the TAP1 and TAP2 genes have been described,^{4,5} and it is possible that differences in human TAP gene products could cause alterations in the peptides presented by class I molecules.

Patients and methods

PATIENTS AND CONTROLS

Fifty eight unrelated white patients with BD, attending the Department of Internal Medicine of the Hospital Virgen del Rocío were included in the study. These patients were diagnosed and classified according to the criteria proposed by the Behçet's Disease Research Committee of Japan.⁶ Their mean age was 41.0 years, and the mean duration of their BD 12.4 years. Fifteen had the 'complete' type of BD showing all four major symptoms of the disease (ocular lesions, oral aphthae, skin lesions and genital ulcerations); the remaining 43 had the 'incomplete' type, showing three major symptoms or ocular symptoms, plus another major symptom and two minor symptoms. Thirty three patients had typical ocular lesions such as recurrent iridocyclitis, hypopyon iritis, retinochoroiditis, or retinal vasculitis. One hundred and sixteen ethnically matched unrelated healthy white subjects served as controls.

HLA CLASS I TYPING

HLA-class I typing was performed on peripheral blood lymphocytes, by the standard NIH technique for the microlymphocytotoxicity test.

DNA EXTRACTION, HLA-DRB1, HLA-DQB1, AND TAP TYPING

Genomic DNA was extracted from 100 μ l of peripheral whole blood using short proteinase K digestion.⁷ HLA class II DNA typing was performed in all subjects by oligotyping after DNA amplification by the polymerase chain reaction (PCR),⁸ as described previously.⁹ Amplification products were blotted onto a

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nylon membrane and hybridised with digoxigenin-labelled probes.^{9,10} Alleles at the TAP1 and TAP2 loci were assigned according to the presence of particular combinations of coding dimorphisms, identified using ARMS (amplification refractory mutation system)-PCR,⁵ which allows detection of polymorphism at positions 333 (Ile or Val) and 637 (Asp or Gly) for TAP1; and at positions 379 (Ile or Val), 565 (Ala or Thr), and 665 (Ala or Thr) for TAP2. Alleles were defined by haplotype association of these dimorphic variants: TAP1A = Ile-333/Asp-637, TAP1B = Val-333/Gly-637, and TAP1C = Val-333/Asp-637; TAP2A = Val-379/Ala-565/Thr-665, TAP2B = Val-379/Ala-565/Ala-665, and TAP2C = Ile-379/Ala-565/Thr-665. Because these alleles are defined by different combinations of single amino acid substitutions, it is not always

possible to assign them unambiguously. For example, at TAP1 the compound heterozygote Val-333/Ile-333 and Asp-637/Gly-637 could be interpreted as a genotype including the 1C and the 1D alleles, but the former is rare and the latter not observed at all in previous studies.⁵ Therefore, the alternative possible interpretation of this combination as a heterozygote TAP1A/TAP1B would be much more likely. The same is true for TAP2 when Val-379/Ile-379 and Thr-665/Ala-665 are present: two interpretations are possible if Ala-565 is found (we were not able to find Thr-565 in any subject)—TAP2B/TAP2C or TAP2A/TAP2H; as TAP2H is an extremely unusual allele, the former possibility is much more likely. We therefore felt it reasonably safe to assume TAP1A/TAP1B and TAP2B/TAP2C, respectively, in such ambiguous cases.

Table 1 HLA-DR and HLA-DQ antigenic frequencies among patients with Behçet's disease (BD) (n = 58) and healthy controls (n = 116)

HLA	BD patients		Controls	
	(No)	(%)	(No)	(%)
DR1	10	17.2	27	23.3
DR2	8	13.8	28	24.1
DR3	11	19.0	28	24.1
DR4	14	24.1	26	22.4
DR11	20	34.5	31	26.7
DR12	1	1.7	3	2.6
DR13	15	25.9	28	24.1
DR14	3	5.2	10	8.6
DR7	19	32.8	30	25.9
DR8	3	5.2	6	5.2
DR9	0	—	2	1.7
DR10	2	3.4	4	3.4
DQ5	13	26.5	45	38.7
DQ6	17	34.7	47	40.5
DQ2	24	49.0	51	44.0
DQ7	20	40.8	35	30.2
DQ8	5	10.2	16	13.8
DQ9	6	12.2	6	5.1
DQ4	3	6.1	11	5.8

Table 2 Phenotype frequency of TAP polymorphisms in patients with Behçet's disease (BD) (n = 58) and controls (n = 116)

TAP	BD patients		Controls	
	(No)	(%)	(No)	(%)
TAP 1				
A	58	100	109	93.9
B	14	24.1	31	26.7
C	0*	—	14	12.1
TAP2				
A	48	83.8	91	78.5
B	29	50.0	67	57.8
C	13	22.4	28	24.1

* $p_{\text{corr}} < 0.05$ compared with controls.

Table 3 Phenotype frequency of TAP polymorphisms in HLA-DQB1*0501-positive and DQB1*0501-negative patients with Behçet's disease (BD) (n = 58) and controls (n = 116)

TAP	BD patients				Controls			
	DQB1*0501+ (n = 13)		DQB1*0501- (n = 45)		DQB1*0501+ (n = 44)		DQB1*0501- (n = 72)	
	(No)	(%)	(No)	(%)	(No)	(%)	(No)	(%)
TAP1								
A	13	100.0	45	100.0	42	95.5	67	93.0
B	7	53.9	7	15.5	14	31.8	17	23.6
C	0	—	0	—	4	9.1	10	13.9
TAP2								
A	10	76.9	38	84.4	32	72.7	59	81.9
B	12	92.3*	17	37.8	25	56.8	42	58.3
C	3	23.1	10	22.2	13	29.6	15	20.8

$p_{\text{corr}} < 0.02$ compared with DQB1*0501-negative BD patients.

STATISTICAL METHODS

Frequency comparisons were made by χ^2 analysis with Yates' correction or by Fisher's exact test, when appropriate. Relative risks (RR) were calculated according to the modified method described by Haldane.¹¹ The level of significance was 0.05 and the p value corrected (p_{corr}) as suggested by Svegaard *et al*,¹² in all cases. Delta values (Δ) were calculated in the analysis of linkage disequilibrium between two loci.

Results

Phenotype frequencies in individuals with BD were compared with ethnically matched controls. As expected, a statistically significant higher frequency of HLA-B51 was observed in BD patients (21 of 58 patients; 36.2%) compared with the control population (18 of 116 individuals; 15.5%) ($p < 0.001$, $RR = 3.1$). No statistically significant differences were found in the frequencies of HLA class II alleles in BD patients compared with the control group (table 1).

Table 2 shows the frequencies of TAP1 and TAP2 alleles in both groups. TAP1C was negatively associated with the disease: it was absent in BD patients, but present in 14 of 116 control subjects (12.1%) ($p_{\text{corr}} < 0.05$, $RR = 0.06$). Two of 18 HLA-B51 positive controls were typed as TAP1C (data not shown).

When different combinations of HLA class II antigens and TAP alleles were analysed, the frequency of DQB1*0501/TAP2B was found to be increased in patients compared with healthy controls (table 3): 12 of 13 BD patients (92.3%) positive for the DQB1*0501 allele bore TAP2B, whereas this combination was present in only 25 of 44 controls (56.8%). This increase can be explained by the existence of a linkage disequilibrium between the alleles in the BD patients ($\Delta = 0.095$, $p_{\text{corr}} < 0.02$), which was not observed in the control group ($\Delta = -0.0034$, $p > 0.05$). This linkage disequilibrium was even stronger in patients with ocular lesions: all nine BD patients positive for HLA-DQB1*0501 bore the TAP2B allele ($\Delta = 0.132$, $p_{\text{corr}} < 0.04$). It was not possible

to state if there was an association between HLA-B51 and TAP2B (or DQB1*0501) in patients and controls because the sample size was too small to allow statistical analysis (three individuals HLA-B51/DQB1*0501 in the patient group and four in the control group).

Discussion

The results of this study agree with previous evidence that susceptibility to BD is influenced by genetic factors mapped within the HLA gene region, B51 being the HLA genetic marker most strongly correlated with disease susceptibility. The results also confirm the strong association of the ocular type of BD with HLA-B51 specificity. No HLA class I antigen other than B51 was shown to be associated with BD, indicating that genetic predisposition to the disease is mainly controlled by HLA-B51 or by some defect(s) in a gene around the HLA-B region in linkage disequilibrium with B51. However, as this association was not complete, additional genetic or environmental factors may be implicated in pathogenesis of Behçet's disease.

The transmembrane domains of TAP1 and TAP2 are thought to form a pore through which antigenic peptides are translocated from the cytosol to the ER,¹³ and this transport seems to be influenced by either the sequence of the peptide, or the polymorphism of the TAP subunits.⁵ The absence of TAP1C in the population of BD patients studied may be explained in terms of this function of TAP molecules: individuals possessing the TAP1C allele may be presumed to have a reduced ability to translocate some peptides involved in the development of BD and thus derive protection from the disease. The absence of TAP1C in BD patients does not seem to be attributable to the increased number of HLA-B51 individuals in this group, or to the existence of a hypothetical linkage disequilibrium between HLA-B51 and TAP1C in the Spanish population, because we found several HLA-B51/TAP1C subjects in the control population. However, the size of the sample was too small for statistical analysis. We cannot exclude the possibility that some BD patients typed as TAP1A/TAP1B were TAP1C/TAP1D, but this seems unlikely because TAP1D has not been observed at all in previous studies.⁵ The same is likely to be true for healthy controls.

Studies performed in different populations of patients with BD have shown a decrease in the frequency of HLA-DQB1*0501 among these patients.² The finding of a linkage disequilibrium between HLA-DQB1*0501 and TAP2B in our patients may indicate that the gene conferring susceptibility to BD is

inherited in association with this haplotype in the population studied, and raises the possibility that its inheritance as an extended haplotype that includes HLA-DQB1*0501 and TAP2B may apply more generally also. This linkage disequilibrium was not observed in our ethnically matched control population; however, in the UK population TAP2B/DR1 (DQB1*0501 has been observed.¹⁴ An alternative explanation is that DQB1*0501 and TAP2B are not necessarily in haplotypic association but may act as trans cofactors in BD. From a practical point of view, this result provides an additional tool to identify individuals at greatest risk of the disease.

The biological mechanisms underlying the effect on disease susceptibility of the TAP2B allele in combination with the DQ5 antigen, and the protection conferred by TAP1C against Behçet's disease are still speculative and require further study. Elucidation of the constraints for peptide selection imposed by TAP may improve prediction of the epitopes likely to be derived from a particular antigen of interest.

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- Ohno S, Anasuma T, Sugiura S, Wakisaka A, Aizawa M. HLA B51 and Behçet's disease. *JAMA* 1978; **240**: 529.
- Mizuki N, Inoko H, Tsuji K, Ding T, Ohno S. Behçet's disease. In: Tsuji K, Aizawa M, Sasazuki T, eds. *HLA 1991*, vol 1. Oxford: Oxford University Press, 1992; 775-80.
- Trowsdale J, Hanson I, Mockridge I, Beck S, Townsend A, Kelly A. Sequences encoded in the class II region of the MHC related to the 'ABC' superfamily of transporters. *Nature* 1990; **348**: 741-4.
- Colonna M, Bresnahan M, Bahram S, Strominger J, Spies T. Allelic variants of the human putative peptide transporter involved in antigen processing. *Proc Natl Acad Sci USA* 1992; **89**: 3932-6.
- Powis S B, Tonks S, Mockridge I, Kelly A P, Bodmer J, Trowsdale J. Alleles and haplotypes of the MHC-encoded ABC transporters TAP1 and TAP2. *Immunogenetics* 1993; **37**: 373-80.
- Mizushima Y. Recent research into Behçet's disease in Japan. *Int J Tissue React* 1988; **10**: 59-65.
- Kawasaki E. Sample preparation from blood cells, and other fluids. In: Innis M, Gelfand D, Sninsky J, White T, eds. *PCR protocols: A guide to methods and applications*. San Diego: Academic Press, 1990; 148.
- Sharf S J, Long C M, Ehrlich H A. Sequence analysis of the HLA-DRB and HLA-DQB loci from three pemphigus vulgaris patients. *Hum Immunol* 1988; **22**: 61-9.
- Yélamos J, García Lozano J R, Moreno I, et al. Association of HLA-DR4-Dw15 (DRB1*0405) and DR10 with rheumatoid arthritis in a Spanish population. *Arthritis Rheum* 1993; **36**: 811-4.
- Yélamos J, García-Lozano J R, Moreno I, Ramero M, García A, Sánchez B. Frequency of HLA-DPB1 alleles in a Spanish population. Their contribution to rheumatoid arthritis susceptibility. *Eur J Immunogen* 1994; **21**: 91-8.
- Haldane J B S. The estimation and significance of the logarithm of a ratio of frequencies. *Ann Hum Genet* 1956; **20**: 309-11.
- Svejgaard A, Ryder P. HLA and disease associations: detecting the strongest association. *Tissue Antigens* 1994; **43**: 18-27.
- Monaco J J. A molecular model of MHC class I-restricted antigen processing. *Immunol Today* 1992; **13**: 173-9.
- Donn R P, Davis E J, Thomson W, Hillarby M C, Ollier W E R. TAP genes associations in UK caucasoid. *Eur J Immunogen* 1994; **21**: 159-67.