

Association of MICA Alleles and HLA-B51 in Italian Patients with Behçet's Disease

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ABSTRACT. Objective. To evaluate the distribution of the MHC class I chain related gene A transmembrane (MICA-TM) alleles in Italian patients with Behçet's disease (BD), and to investigate the relative contribution of MICA alleles and HLA-B51 in the susceptibility and specific clinical features of BD.

Methods. A total of 69 consecutive Italian patients who satisfied the International Study Group criteria for BD were followed at rheumatology, ophthalmology, and neurology units during a 3 year period (1997–99). We selected 130 healthy subjects from the same geographic areas as controls. All patients and controls were examined for MICA microsatellite polymorphisms using polymerase chain reaction. Serological HLA class B51 typing was performed by a standard microlymphocytotoxicity technique.

Results. A strong association with HLA-B51 was observed in patients with BD (OR 5.7, 95% CI 2.8–11.3). The MICA-TM allele A6, in linkage disequilibrium with HLA-B51, was only slightly increased in patients compared to controls (60.9% vs 50.8%; $p = \text{NS}$). No significant associations between HLA-B51 or MICA-TM alleles and clinical subgroups, particularly central nervous system or eye involvement, were found.

Conclusion. HLA-B51 is the most important susceptibility gene in BD. Association with MICA-A6, when it exists, is secondary to the strong linkage disequilibrium with HLA-B51. (J Rheumatol 2001;28:1867–70)

Key Indexing Terms:
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Behçet's disease (BD) is a multisystem inflammatory disease of unknown cause, characterized by recurrent oral aphthous ulcers, genital ulcers, uveitis, and skin lesions. Vasculitis is the pathological lesion common to most of the clinical manifestations of BD^{1,2}.

BD is a polygenic disease whose multiple genetic factors, in combination with undefined environmental risk factors

such as infectious agents, are probably of importance in determining susceptibility to the disease. To date, the strongest genetic association identified in BD has been with HLA-B51 allele¹⁻⁵. This association is shared by different ethnic groups, in particular in the countries along the ancient "Silk Route," where BD is found with the highest prevalence⁵.

The major histocompatibility complex (MHC) class I chain related gene A (MICA) is a functional gene located between the HLA-B and TNF genes on the short arm of human chromosome 6⁶. The MICA gene is highly polymorphic^{7,8} and is mainly expressed in epithelial cells, keratinocytes, endothelial cells, and monocytes⁹.

Polymorphism in exon 5 is composed of at least 5 alleles (A4, A5, A5.1, A6, and A9) presenting 4, 5, 6, and 9 triplet repeats of (GCT/AGC) in the transmembrane (TM) region of the MICA gene¹⁰. Further, a novel allele with 10 GTC repetitions (A10) has recently been reported¹¹.

In 1997 Mizuki, *et al* reported a strong association of MICA-A6 allele in Japanese patients with BD¹⁰. In this study MICA-A6 was in strong linkage disequilibrium with HLA-B51 and appeared to be more closely associated with BD than HLA-B51 itself. Subsequent studies in different populations have reported different results confirming the association between HLA-B51 and MICA-A6 in Greek and Palestinian or Jordanian patients^{12,13}, but not in Spanish patients¹⁴.

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We investigated the distribution of A4, A5, A5.1, A6, and A9 MICA-TM alleles in 69 Italian patients with BD and 130 ethnically matched healthy controls. We also investigated the relative contribution of MICA alleles and HLA-B51 in the susceptibility and specific clinical features of BD.

MATERIALS AND METHODS

Study population. Our subjects were consecutive Italian patients with BD followed at the Bologna, Ferrara, Milan, Potenza, Prato, Reggio Emilia, and Trento rheumatology, ophthalmology, and neurology units during a 3 year period (1997–99) who satisfied the International Study Group criteria for BD¹⁵.

The study cohort comprised 69 patients. The control group consisted of 130 healthy subjects who were unrelated blood donor volunteers. All subjects were Caucasians residing in Italy for at least one generation. No ethnic differences were present between patients and controls, and none were of Jewish background. Informed consent was obtained from patients and controls before the study.

HLA class I typing. Serological HLA class I typing was performed by the standard microlymphocytotoxicity technique, using peripheral blood lymphocytes¹⁶; 56 of the 69 patients were typed for HLA-B51 allele.

Triplet repeat polymorphism in the TM region of the MICA gene. The TM region of the MICA gene was amplified using polymerase chain reaction (PCR) primers and conditions as described¹⁰. Aliquots of the PCR product were denatured for 5 min at 96°C, mixed with formamide containing stop buffer, electrophoresed onto 7.5% polyacrylamide gel containing 7 M urea in TBE buffer (Tris-borate 0.089 M, EDTA 0.002 M, pH 8.0), and run overnight under 700 V constant current at room temperature. The gel was then stained with silver (Biorad; Hercules, CA, USA). To determine the number of repeat units of the microsatellite alleles a panel of reference samples was previously analyzed using an automated DNA sequencer (Applied Biosystems model 310 Genetic Analyzer). The PCR was carried out by the same procedure as described, except for the addition of 0.5 μM fluorescent dNTPs (R110 dUTP; Applied Biosystems). The amplified and denatured products were run with the size standard marker GS350 ROX; the fragment sizes were determined automatically using GeneScan 672 Software (Applied Biosystems) and MICA-A4, -A5, -A5.1, -A6, -A9 alleles were assigned. These reference samples were then used as size standards and loaded into polyacrylamide gels among the unknown samples, allowing assignment of the alleles by comparison.

Statistical analysis. Statistical analysis was done using the SPSS statistical package (SPSS Inc., Chicago, IL, USA). The frequencies of the alleles and genotypes among patients and controls were compared by chi-squared test and Fisher's exact probability test. Odds ratios (OR) and 95% confidence intervals were calculated. OR was estimated by the method of Haldane if required. Corrected p values were calculated by multiplying p by the number of MICA-TM alleles.

RESULTS

Table 1 shows the clinical and demographic characteristics of 69 Italian patients with BD. The phenotype frequencies of the MICA-TM alleles are shown in Table 2. No significant associations were found between MICA alleles and BD. The frequency of MICA-A6 was higher in BD patients than in controls, although the difference was not statistically significant. Of 69 patients with BD, 12 were homozygous or heterozygous for the MICA-A9 allele (17.4%), while 45 of 130 controls had the MICA-A9 allele (35.6%). Thus, the MICA-A9 allele was found to be negatively associated with BD, although the significance was lost after correction of the p value ($p = 0.02$, $p_{\text{corr}} = 0.10$).

Table 1. Demographic and clinical features of 69 Italian patients with Behçet's disease.

	N	%
Female/male	28/41	41/59
Mean age at disease onset ± SD, yrs	31 ± 12	
Mean disease duration ± SD, yrs	12 ± 9	
Oral ulcer	69	100
Genital ulcer	40	58.0
Erythema nodosum	23	33.3
Papulopustular lesions	54	78.3
Eye lesions	50	72.5
Arthritis	32	46.4
Deep vein thrombosis	15	21.7
Central nervous system involvement	20	29.0
Epididymitis	3	4.3
Positive pathergy test*	7	38.9

*Pathergy test was performed on 18 patients.

Table 2. Phenotype frequencies of MICA and HLA-B51 alleles among patients with Behçet's disease and controls.

	Healthy Controls, N = 130 (%)	Behçet's Disease, N = 69 (%)	p	OR (95% CI)
MICA allele				
A4	28 (21.5)	16 (23.2)	NS	1.1 (0.6–2.2)
A5	27 (20.8)	15 (21.7)	NS	1.1 (0.5–2.2)
A5.1	60 (45.2)	35 (50.7)	NS	1.2 (0.7–2.2)
A6	66 (50.8)	42 (60.9)	NS	1.5 (0.8–2.7)
A9	45 (34.6)	12 (17.4)	0.02**	0.4 (0.2–0.8)
HLA-B51*	25 (19.2)	31 (57.4)	0.0001	5.7 (2.8–11.3)

*54/69 patients with BD were typed for HLA-B51. ** $p_{\text{corr}} = 0.10$.

No specific association was found between MICA-TM alleles and any of the disease features evaluated in Table 1, including ocular, cerebral, or cutaneous involvements (data not shown). Fifty-six patients were typed for HLA-B51; 31 (57.4%) were HLA-B51 positive, compared with 25 of the 130 controls ($p = 0.0001$, OR 5.7, 95% CI 2.8–11.3) (Table 2). The association between HLA-B51 and BD clinical findings defined in Table 1 was evaluated in the 56 patients. We found only a trend of higher frequency of HLA-B51 in patients with cutaneous manifestations compared to those without (90.9% vs 69.6%; $p = 0.07$).

A strong association was found between HLA-B51 and MICA-A6 in both the controls and patients with BD (Table 3). All the B51 positive controls (25/25) and 24/31 (77.4%) of the B51 positive patients possessed MICA-A6, revealing a strong linkage disequilibrium between MICA-A6 and HLA-B51.

We examined MICA-A6 allele association with BD, stratifying for the possible confounding effect of HLA-B51.

Table 3. Association of MICA-A6 and HLA-B51 in controls and patients with Behçet's disease.

	HLA-B51			
	Controls N = 130 (%)		Patients N = 54 (%)	
	Present	Absent	Present	Absent
MICA-A6				
Present	25 (100)	41 (39)	24 (77.4)	8 (34.8)
Absent	0	64 (61)	7 (22.6)	15 (65.2)
OR	79.3		6.4	
p	0.0001		0.002	
95% CI	44.7–148.4		1.9–21.4	

No significant associations were observed in this analysis (data not shown). When we examined HLA-B51 association with BD, stratifying for the possible confounding effect of MICA-A6, a statistically significant association of HLA-B51 with BD was found in both the MICA-A6 positive and negative subgroups (Table 4).

DISCUSSION

Hyperfunction of neutrophils and both phenotypical and functional abnormalities of $\gamma\delta$ T cells are characteristics of BD, and probably have a central role in the pathogenesis of this disease^{1,2,17,18}. HLA-B51 and MICA are strictly correlated with these 2 abnormalities. Various evidence suggests that HLA-B51 molecules by themselves and/or the related gene products may be partly responsible for the excessive function of neutrophils^{19,20}. Although the function of the MICA molecule is not yet fully understood, MICA genes are considered cell stress response genes²¹, and it has been suggested that MICA may play an important role in the stimulation of $\gamma\delta$ T cells through a stress induced mechanism. The report that MICA molecule is recognized by intestinal epithelial $\gamma\delta$ T cells supported this hypothesis²².

We found no association between MICA-TM alleles and BD in our study population. Only a nonsignificant increase of the frequency of MICA-A6 was observed in the patients with BD. As reported in Italian populations, we found a strong association of patients with HLA-B51²³. A strong

linkage between MICA-A6 and HLA-B51 was observed in both patients and healthy controls (Table 3). Stratification analysis of the MICA-A6 patients with BD for the possible confounding effect of HLA-B51 and vice versa (Table 4) confirmed that HLA-B51 allele was the major susceptibility gene for BD in our Italian population.

The association of MICA-A6 with BD in a Japanese population was described by Mizuki, *et al*¹⁰, who found this allele present at a significantly higher frequency in patients with BD than in controls. They suggested the possibility of a primary association of BD with MICA-A6 rather than HLA-B51, because MICA-A6 was found in all HLA-B51 positive patients and a significant fraction of patients who were B51 negative.

Subsequently 2 other studies showed an association between BD and MICA-A6 in Greek and Palestinian or Jordanian patients^{12,13}. In these 2 studies MICA-A6 and HLA-B51 were in strong linkage disequilibrium and the association with HLA-B51 was stronger than that with MICA-A6.

However, a study from Spain¹⁴ showed no association of MICA-TM alleles with BD. A slight nonsignificant increase of MICA-A6 and a strong association with HLA-B51 were reported in Spanish patients.

In addition to the triplet repeat (GTC/AGC) polymorphism identified in the MICA transmembrane region, 20 external domain MICA alleles have been described. Recently, Mizuki *et al*²⁴ studied the association of these external domain alleles with BD and observed a strong association with MIC-A*009. MIC-A*009 is one of the 4 external domain alleles that contain the A6 transmembrane repeat, as do MIC-A*003, *004, and *008. Therefore this association was expected in Japanese patients. However, stratification and linkage analyses between MIC-A*009 and HLA-B51 clearly indicated that HLA-B51 is the major susceptibility gene in BD.

Thus even if we did not find a significant association with MICA-A6, as in the Spanish patients¹⁴, our data are in agreement with studies indicating HLA-B51 as the most important susceptibility gene in BD in an Italian population and association with MICA-A6 secondary to the strong linkage disequilibrium with HLA-B51.

We also evaluated if MICA alleles or HLA-B51 antigen were associated with specific clinical findings. Only a trend of higher frequency of HLA-B51 was observed in patients with cutaneous manifestations. No associations with patients with more severe disease, in particular with central nervous system or eye involvement, were observed. Thus in Italian patients HLA-B51 was a marker of disease susceptibility, but it was not associated to specific clinical features of BD.

Italy's general population has an HLA-B51 prevalence greater than 10%²³. It is one of the countries with the highest prevalence of this allele⁵. HLA-B51 seems to be one of the

Table 4. Association of HLA-B51 with Behçet's disease after stratification for the effect of MICA-A6.

	MICA-A6 Negative		MICA-A6 Positive	
	Controls, N = 64 (%)	Patients, N = 22 (%)	Controls, N = 66 (%)	Patients, N = 32 (%)
HLA-B51				
Present	0	7 (31.8)	25 (37.9)	24 (75.0)
Absent	64 (100.0)	15 (68.2)	41 (62.1)	8 (25.0)
OR	62.4		49.2	
p	0.0001		0.001	
95% CI	27.1–134.3		1.92–12.62	

primary risk factors for BD, and an attractive hypothesis is that BD was spread in parallel with the distribution of its associated allele by population movement between the Mediterranean and Asia along the Silk Route. However, since around 43% of Italian patients were HLA-B51 negative, a contributory role of some other genes and/or environmental factors is likely.

We evaluated HLA-B51 and MICA-TM allele associations in 69 Italian patients with BD. We found a strong association with HLA-B51, but only a nonsignificant slight increase of MICA-A6 in patients with BD compared to ethnically matched healthy controls. A strong linkage disequilibrium in patients and controls was observed between HLA-B51 and MICA-A6 allele. Thus, the possibility that the MICA gene is primarily involved in the pathogenesis of BD can be excluded, supporting the hypothesis of a primary association of BD with HLA-B51.

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