EXTENDED REPORT

Association of the MIC-A gene and HLA-B51 with Behçet's disease in Arabs and non-Ashkenazi Jews in Israel

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Ann Rheum Dis 2002;**61**:157–160

Background: Behçet's disease is known to be strongly associated with HLA-B51 in many different ethnic groups. Recently, it was suggested that MIC-A (major histocompatibility complex class I related gene A) is the pathogenic gene after strong association was found between the MIC-A A6 allele of the transmembrane region and the disease in Japanese and Greek patients, although in Greek patients this association was found to be due to linkage disequilibrium with HLA-B51.
Objectives: To investigate microsatellite polymorphism in Arab and non-Ashkenazi Jewish (NAJ) patients in Israel, to determine whether this association occurs in these groups with Behçet's disease, and elucidate the associated HLA allele of the disease.
Methods: Forty four Israeli patients with Behçet's disease, including 20 Arabs and 24 NAJ, and 130 ethnically matched healthy controls were examined for MIC-A microsatellite polymorphism of the transmembrane region using polymerase chain reaction, autoradiography, and sequence analysis.
Results: The MIC-A A6 allele was significantly more frequent in the Arab patient group (19/20 (95%)) than in healthy Arab controls (25/42 (60%)) (p_{corr}=0.015, OR=12.92), but not in the NAJ patients (16/24 (67%)) compared with NAJ healthy controls (48 /88 (55%)) (p_{corr}=1.02, OR=1.667). In stratification analysis of the Arab subgroup, on the confounding effect of MIC-A A6 on HLA-B51 association and vice versa, Behçet's disease was distinctly associated only with HLA-B51.
Conclusions: These results imply strong association between the MIC-A A6 allele and the disease in Israeli Arabs, but not in Israeli NAJ patients. The stratification analysis indicates that this association results secondarily from a strong linkage disequilibrium with HLA-B51, and the real disease susceptibility gene which plays a part in the development of Behçet's disease is most probably the HLA-B51 allele itself.

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Accepted 2 July 2001

Behçet's disease (BD) is a chronic systemic inflammatory disorder characterised by a course of remissions and exacerbations of unpredictable frequency and duration. The clinical characteristics include involvement of mucous membranes with oral and genital ulcers, ocular and skin lesions, as well as central nervous system, joint, vascular, and gastrointestinal manifestations.[1–4] The disease exists world wide but is most prevalent in the Far East, (Japan, China, and Korea), along the Silk Route, and in the countries of the Mediterranean Basin, including Israel.[1,3] The cause and pathogenesis of BD are still unknown, but the onset of the disease is believed to be triggered by external environmental factors in people with a particular genetic susceptibility.[5] HLA-B51 is the genetic marker most strongly associated with BD in different populations, with relative risk ranging from 1.38 to 20.70.[6–10] However, it is still unknown whether HLA-B51 participates directly in the pathogenesis of the disease or is only associated with it because of linkage disequilibrium with a nearby gene.[11,12]

Recently, the major histocompatibility complex (MHC) class I chain related gene A (MIC-A) has been proposed as a candidate gene for BD susceptibility.[13] The MIC-A gene belongs to the MHC class I related genes and is located 46 kb centromeric to the HLA-B gene on chromosome 6.[13] Its complete nucleotide sequence and exon intron organisation have been previously reported.[14] The gene is mainly expressed in epithelial cells, fibroblasts, endothelial cells, and monocytes.[15–17] Its function is still unknown, but its amino acid sequence suggests that it may bind peptides or other short ligands and participate in antigen presentation or T cell recognition.[15]

During nucleotide sequence analysis of the MIC-A gene Mizuki *et al*[13] found a triplet repeat microsatellite polymorphism of (GCT/AGC) in exon 5, encoding for the transmembrane (TM) region of the gene product. The polymorphism in exon 5 is composed of at least six alleles named A4, A5, A5.1, A6 "old", A6 "new", and A9, representing four, five, six, and nine triplet repeats of (GCT/AGC) respectively.[13] The A5.1 allele represents an insertion (G) between the second and third triplet repeats resulting in a premature stop codon. The A6 "new" stands for a C/T substitution at position 991 in some A6 alleles, which gives rise to a Cys/Arg amino acid change.[13,18]

The polymorphism in exon 5 of the MIC-A gene was studied in several populations. In the Japanese population the A6 allele was found in a significantly higher frequency in patients than in controls. In this study all HLA-B51 positive patients with BD carried the A6 allele, which was also present in some HLA-B51 negative patients.[13] The same results were found in a Greek population, but Yabuki *et al* raised the possibility that the higher frequency of the A6 allele resulted from linkage disequilibrium with the HLA-B gene.[19] In a study of patients with BD in Spain, the authors found that the A6 allele frequency was slightly increased (not significantly) and concluded that in Spain HLA-B51 is more closely associated with BD susceptibility than are MIC-A-TM genes.[20] Israel has

Abbreviations: BD, Behçet's disease; MIC-A, major histocompatibility complex class I related gene A; NAJ, non-Ashkenazi Jews; PCR, polymerase chain reaction; TM, transmembrane

Table 1 Phenotype frequencies of microsatellite polymorphism in the transmembrane region (exon 5) of the MIC-A gene in Arab patients with BD and control subjects

Microsatellite allele	Amplified product (bp)	Patients (%) (n=20)	Controls (%) (n=42)	OR	p Value	p_{corr}
A4	179	3 (15)	12 (29)			
A5	182	4 (20)	14 (33)			
A5.1	183	4 (20)	6 (14)			
A6	185	19 (95)	25 (60)	12.92	0.003	0.015
A9	194	5 (25)	13 (31)			

a diverse ethnic population, including Ashkenazi Jews from Central and Eastern Europe, non-Ashkenazi Jews (NAJ) from Northern Africa and Middle East countries, and the local Arab population. Therefore, the MIC-A study of patients with BD in Israel is of particular interest.

In our study, to determine whether MIC-A is associated with BD in different ethnic groups in Israel, we examined the distribution of A4, A5, A5.1, A6 (old only), and A9 MIC-A-TM alleles, in 44 patients with BD, 20 of them Arabs and 24 NAJ, and in 130 ethnically matched healthy unrelated controls.

PATIENTS AND METHODS

Patients and controls

Forty four Israeli patients with BD, of whom 20 were Israeli Arabs and 24 were NAJ originating from Morocco (n=7), Tunisia (n=2), Yemen (n=3), Turkey (n=3), and Iraq (n=9), were included. All the patients fulfilled the international study group diagnostic criteria for BD.[21] All were followed up in the day care centre of the internal medicine division of Hadassah Hospital, Jerusalem. One hundred and thirty healthy control subjects (42 Israeli Arabs and 88 NAJ originating from Iraq (n=30), Morocco and Tunisia (n=43), Turkey and Yemen (n=15)) matched to the patients by ethnic origin, and unrelated to each other or to the patients, were also included.

HLA class I typing

The patients were previously typed for HLA class I by a standard microlymphocytotoxicity technique.[22] The controls were typed for HLA-B51 (positive or negative) by Micro SSP HLA DNA typing trays (One Lambda Inc CA, USA), according to the manufacturer's protocol.

Analysis of triplet repeats polymorphism in the TM region of the MIC-A gene

Genomic DNA from patients and controls was extracted from peripheral blood with the PureGene kit (GENTRA System Inc). Polymerase chain reaction (PCR) primers flanking the TM region were designed as described previously.[13] A total of 100–200 ng of high molecular weight DNA was subjected to PCR amplification of the TM region in the MIC-A gene. The amplification reaction was carried out in a final volume of 50 μl containing 5 μl of 10×PCR buffer (Boehringer), 0.5 mM each of dATP, dDTP, dGTP, dCTP, with trace [^{32}P]dGTP, 6 μg/ml of each primer, and 1.25 units of Taq polymerase (Boehringer). PCR amplification was carried out in a GMG Research minicycler under the following conditions: one cycle of denaturation at 94°C for two minutes followed by 35 cycles of denaturation at 94°C for one minute, annealing at 55°C for one minute, and extension at 72°C for two minutes. To determine the number of triplet repeats, the PCR samples were electrophoresed on 5% polyacrylamide gels containing 8 M urea. Gels were dried and then autoradiographed for detection of the five alleles. DNA samples from homozygous subjects were subjected to sequence analysis to verify the length of the different alleles. Sequence was automatically performed by an ABI 310 sequencer (Perkin-Elmer). Analysis was done by sequence analysis software (PE).

Statistical analysis

Allele and phenotype frequencies were estimated by direct counting. The significance of the distribution of alleles between the patients with BD and controls was tested by the χ^2 method with the continuity correction and Fisher's exact probability test (p test value). The p value was corrected by multiplication by the number of microsatellite alleles, five, in the TM region of the MIC-A gene (p_{corr} value). When one of the cells measured zero, the odds ratio (OR) was calculated by Haldane's modification of Woolf's method.[23]

RESULTS

To examine the association between the microsatellite polymorphism in the TM region of the MIC-A gene and the disease in Israel, we examined 20 Arab patients and 42 matched Arab controls, and 24 NAJ patients and 88 matched NAJ controls. All five distinct alleles, described previously, were found in the patients and controls. Tables 1 and 2 show the phenotype frequencies in the Arab and NAJ groups, respectively. Of 20 Arab patients with BD, 19 had the MIC-A A6 allele in a homozygous or heterozygous pattern (95%), whereas 25/42 (60%) Arab controls had the A6 allele. Of 24 NAJ patients, 16 had the MIC-A A6 allele (67%), whereas 48/88 (55%) NAJ controls had the A6 allele. Thus, in Israel, the

Table 2 Phenotype frequencies of microsatellite polymorphism in the transmembrane region (exon 5) of the MIC-A gene in non-Ashkenazi Jewish patients with BD and control subjects

Microsatellite allele	Amplified product (bp)	Patients (%) (n=24)	Controls (%) (n=88)	OR	p	p_{corr}
A4	179	3 (13)	19 (22)			
A5	182	9 (38)	25 (28)			
A5.1	183	7 (29)	29 (33)			
A6	185	16 (67)	48 (55)	1.667	0.204	1.02
A9	194	4 (17)	27 (31)			

Table 3 Association of BD with MIC-A A6 and HLA-B51 in the Arab subpopulation

	Controls (%) (n=42)	BD (%) (n=20)
MIC-A A6*		
Present	25 (60)	19 (95)
Absent	17 (40)	1 (5)
HLA-B51†		
Present	5 (12)	15 (75)
Absent	37 (88)	5 (25)

*OR=12.92; p=0.003; 95% CI 1.58 to 105.84;
†OR=22.2; p=0.00001; 95% CI 5.6 to 87.99.

Table 4 Association of MIC-A A6 with BD stratified for the effect of HLA-B51

	Absence of HLA-B51		Presence of HLA-B51	
MIC-A A6	Controls	Patients with BD	Controls	Patients with BD
Present	21	5	5	14
Absent	16	0	0	1
OR	12.772		0.879	
p Value	0.077		0.75	
95% CI	0.435 to 160.781		0.03 to 24.992	

Table 5 Association of HLA-B51 with BD stratified for the effect of MIC-A A6

	Absence of MIC-A A6		Presence of MIC-A A6	
HLA-B51	Controls	Patients with BD	Controls	Patients with BD
Present	0	1	5	14
Absent	16	0	21	5
OR	99		11.76	
p Value	0.058		0.0003	
95% CI	1.396 to 7202.63		2.865 to 42.269	

MIC-A A6 allele was found to be strongly associated with BD only in Arab patients (OR=12.92; p=0.003; p_{corr}=0.015), but not in the NAJ patients (OR=1.667; p=0.204; p_{corr}=1.02). This difference between the two ethnic groups is unrelated to the general A6 allele frequency, because a comparison of both control groups yielded insignificant results (OR=1.226; p=0.366).

No specific association was found between the MIC-A A6 allele and any of several disease features tested, including uveitis, erythema nodosum, arthritis, or vascular manifestations (data not shown). As summarised in table 3, the HLA-B51 allele was found in 5/42 (12%) Arab controls and in 15/20 (75%) Arab patients with BD, whereas the MIC-A A6 allele was found in 25/42 (60%) Arab controls and in 19/20 (95%) Arab patients with BD. Statistical analysis of these results showed that the association of MIC-A A6 with BD produced an OR of 12.92 (p=0.003), whereas HLA-B51 produced an OR of 22.2 (p=0.00001).

The MIC-A A6 allele was found in 16/24 (67%) NAJ patients and in 48/88 (55%) NAJ controls. The HLA-B51 allele was found in 19/23 (83%) NAJ patients. HLA status was not studied in the NAJ controls, but we have previously shown that 13% of a healthy NAJ control population carry HLA-B51.[7] Thus in the NAJ BD subgroup there is a strong association between the disease and the HLA-B51 allele, but there is no significant association with the MIC-A A6 allele (OR=1.667; p=0.204).

To determine whether HLA-B51 or MIC-A A6 is the pathogenic gene related to BD, an association of MIC-A A6 with BD stratified for the possible confounding effect of B51 was analysed in the Arab population. No association was seen in this analysis (table 4). In contrast, when an association of B51 with BD stratified for the possible confounding effect of A6 was analysed, a statistically significant association of B51 was found (table 5).

DISCUSSION

The frequency of the HLA-B51 antigen is approximately 60% in patients (of different ethnic groups) with BD.[6–10] However, it has been uncertain whether B51 itself, or another gene in linkage disequilibrium with it, is responsible for the development of BD. Recently, the MIC-A gene located 46 kb centromeric to HLA-B51 has been proposed as a candidate gene for BD susceptibility. A strong association between the MIC-A A6-TM allele and the disease was reported in Japan.[13]

In our study, to clarify whether the real pathogenic gene of BD is HLA-B or MIC-A, we investigated microsatellite polymorphism in the TM region of the MIC-A gene in an ethnically diverse Israeli BD population, including Arabs and NAJ. MIC-A A6 was found to be strongly associated with BD only in the Arab subgroup. Studies performed recently in Spain and Greece also showed conflicting results. Whereas in Greece the same association was found between MIC-A A6 and the disease as in Japan,[19] no such association was found in Spain.[20] A possible explanation for this discrepancy may be that our NAJ group includes subjects of different origins, including Morocco, Tunisia, Iraq, Yemen, and Turkey. It is well known that these various groups have different genetic origins. A separate examination of the NAJ group according to more homogenic genetic subgroups such as Moroccans, Yemenis, etc, might have yielded the same association as in the Arab patients. However, this analysis was not done because of the small number of subjects in each subgroup.

To assess the relative strength of the MIC-A A6 and HLA-B51 association with the disease, we stratified the results of the MIC-A A6 in patients and controls on the possible confounding effect of B51 and vice versa (tables 4 and 5), as described by Svejgaard and Ryder.[23] This analysis clearly suggests that the significant increase of MIC-A A6 in the Arab patients may be explained by linkage disequilibrium with HLA-B51. Moreover, this stratification analysis has shown the same results in Spain[20] and Greece,[19] and also in a study done by Mizuki *et al* on the MIC-A009 allele.[24]

Previous studies using microsatellite mapping have excluded an association between MIC-B genes,[25] and HLA-C genes and BD. The critical region for the disease susceptibility was reported by Ota *et al* to be reduced to a 46 kb segment between the MIC-A gene and the HLA-B gene.[26] Presently, no other open reading frame has been detected in this critical segment except MIC-A and HLA-B.[26] This study, together with the results of the Spanish[20] and Greek[19] populations, and results reported recently by Mizuki *et al*,[27] indicates that the association of the MIC-A gene with the disease results from linkage disequilibrium with HLA-B51. This suggests that the associated HLA allele for BD is the HLA-B gene itself and that the HLA-B51 allele is the major susceptibility gene responsible for the development of the disease. The presence of HLA-B51 negative patients with BD may be explained by the influence of other genetic factor(s) and/or various external environmental or infectious agent(s).

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