Association of MICA alleles with psoriatic arthritis and its clinical forms. A multicenter Italian study

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ABSTRACT

Objective. *Analysis of the association between psoriatic arthritis (PsA) clinical forms and MICA gene transmembrane polymorphisms.*

Methods. *Patients were classified as having peripheral asymmetric oligoarthritis (AO), peripheral symmetric polyarthritis (PA) and spondylitis (SP), or disease combinations (PA/SP, OA/SP). Two hundred and twenty-six patients with PsA were typed for MICA exon 5 microsatellite (TM) by heteroduplex analysis and compared with 225 normal controls.*

Results. *MICA-TM microsatellite typing revealed that, among the different clinical forms of PsA, only the combined PA/SP subset shows a significant positive association with MICA-A9 and a lower frequency of MICA-A4, A5 genotype in PsA patients with a decrease, only in the PA/SP cohort, of all MICA-A5 combinations except MICA-A5, -A9.*

Conclusion. *These results suggest a role for genes within the HLA region in the pathogenesis of PsA, and reinforce the idea that the different forms of PsA may have heterogeneous genetic basis.*

Introduction

Among the genetic factors that confer susceptibility to psoriatic arthritis (PsA), a role seems to be played by genes harbored within the HLA region (1, 2). In particular, it has been reported a significant association of several HLA alleles of class I and II with the clinical forms of the disease. These include the peripheral symmetric (rheumatoid-like) polyarthritis (PA), the asymmetric oligoarthritis (OA) and the spondylitis (SP) form (3), and the combined expressions, while the mutilans form is rarely observed.

More recently, other genes in the HLA region have been investigated and, in particular, a strong positive association has been found between the MICA-A9 allele, which is in linkage disequilibrium with B38 and B39, and PsA (4, 5) notably in the PA form. This association is independent from that of Cw6 – which seems to be determinant in psoriasis (6) – and both alleles, Cw6 and MICA-A9, appear to confer, in the Spanish population, susceptibility to the disease (7), while in the Croatian population only the MICA-A4 allele was found to be present at a significantly higher frequency in the PsA patients than in the controls (8).

We report here a positive association between the combined PA/SP form of PsA and MICA-A9. Genotype analysis has shown that a reduced frequency of MICA-A5 holds in PsA patients when this allele is expressed in combination with the shortest MICA transmembrane allele (MICA-A4).

Materials and methods

Patients and controls

Unrelated Caucasian PsA patients were enrolled in five Rheumatological University Centres (Padua, Rome, Naples, Bari and Cagliari) of peninsular and insular Italy. Patients were classified according to the Moll and Wright criteria (3).

Two hundred and twenty-six (226) unrelated Italian patients with PsA were consecutively recruited: 98 (43%) had PA alone, 53 (23%) patients had isolated OA, 7 (3%) had isolated SP; 50 (22%) had combined PA and SP (PA/SP), and 18 (8%) showed combined OA and SP (OA/SP). Two hundred and twenty-five unrelated healthy subjects derived from the same areas of patients with PsA were enrolled as the control group. All patients and healthy controls included in this study gave their informed consent.

The 226 patients with PsA and the 225 healthy controls were typed for MICA exon 5 microsatellites.

Typing for MICA alleles

DNA samples were typed blindly from the clinical diagnosis. MICA exon microsatellite typing was performed by DNA heteroduplex analysis as previously described (9). Briefly, the following primers were used to amplify exon 5: MICA-FOR: 5'-CCTTTTTTTCAGGGAAAGTGC-3'; MICA-REV: 5'CTTACCATCTCCAGAAACTGC-3'. PCR was performed in a final volume of 50µl by 30 denaturation cycles at 94°C for 30 sec, annealing at 60°C for 30 sec, and extention at 72°C for 30 sec; the final step was carried out for 7

Table I. Frequencies of MICA alleles in patients with PsA.

MICA allele	% of healthy controls (n = 225)	% of patients with PsA (n=226)	% of isolated PA (n=98)	% of isolated OA (n=53)	% of combined PA/SP (n=50)	% of combined OA/SP (n=18)	% of isolated SP (n=7)
A4	30 (68)	36.2 (82)	33.6 (33)	39.6 (21)	30.0 (15)	55.1 (10)	42.8 (3)
A5	31 (69)	20.3 (46)	21.4 (21)	20.7 (11)	14.0 (7)	33.3 (6)	14.2 (1)
A5.1	46 (104)	42.0 (95)	45.9 (45)	45.2 (24)	34.0 (17)	38.8 (7)	28.5 (2)
A6	37 (83)	38.0 (86)	32.6 (32)	45.2 (24)	42.0 (21)	22.2 (4)	71.4 (5)
A9	39 (87)	47.7 (108)	46.9 (46)	41.5 (22)	64.0*(32)	33.3 (6)	28.5 (2)

*p=0.0015 (p_{corr}=0.0075); OR=2.82 (95% CI=1.49-5.33)

Table II. Frequencies of MICA genotypes in patients with PsA.

MICA genotype	% of healthy controls (n=225)	% of patients with PsA (n=226)	% of isolated PA (n=98)	% of isolated OA (n=53)	% of combined PA/SP (n=50)	% of combined OA/SP (n=18)	% of isolated SP (n=7)
A4/A4	2.6 (6)	1.3 (3)	2 (2)	0 (0)	2 (1)	0 (0)	0 (0)
A4/A5	5.7 (13)	0.8* (2)	0 (0)	0 (0)	2 (1)	5.5 (1)	0 (0)
A4/A5.1	7.1 (16)	14.1 (32)	15 (15)	15 (8)	6 (3)	27.7 (5)	14.2 (1)
A4/A6	6.6 (15)	8.4 (19)	10 (10)	5.6 (3)	8 (4)	5.5 (1)	14.2 (1)
A4/A9	8.0 (18)	11.5 (26)	6 (6)	18.8 (10)	12 (6)	16.6 (3)	14.2 (1)
A5/A5	2.2 (5)	1.7 (4)	3 (3)	0 (0)	0 (0)	5.5 (1)	0 (0)
A5/A5.1	9.3 (21)	5.7 (13)	6 (6)	11.3 (6)	0 (0)	5.5 (1)	0 (0)
A5/A6	8.0 (18)	6.2 (14)	5 (5)	7.5 (4)	4 (2)	11.1 (2)	14.2 (1)
A5/A9	5.3 (12)	5.7 (13)	7 (7)	1.8 (1)	8 (4)	5.5 (1)	0 (0)
A5.1/A5.1	6.6 (15)	1.7 (4)	2 (2)	0 (0)	4 (2)	0 (0)	0 (0)
A5.1/ A6	10.2 (23)	8.8 (20)	8 (8)	11.3 (6)	10 (5)	0 (0)	14.2 (1)
A5.1 /A9	12.8 (29)	11.5 (26)	14 (14)	7.5 (4)	14 (7)	5.5 (1)	0 (0)
A6/A6	2.6 (6)	3.0 (7)	1 (1)	7.5 (4)	0 (0)	5.5 (1)	14.2 (1)
A6/A9	9.3 (21)	11.9 (27)	8 (8)	13.2 (7)	22 (11)	0 (0)	14.2 (1)
A9/A9	3.1 (7)	7.0 (16)	11 (11)	0 (0)	8 (4)	5.5 (1)	0 (0)

*p=0.0036 (p_{corr}=0.05); OR=0.14 (95% CI 0.03-0.65)

min at 72°C. Aliquots of the amplified products were mixed with a MICA-A4 amplified DNA and run on a 12% polyacrilamide gel, which was stained with ethidium bromide and photographed by a Kodak EDAS 290 system.

Statistical analysis was carried out by χ^2 test with Bonferroni's correction and p-values were corrected by the number of comparisons made.

Haplotype analysis

To verify whether the associations reported so far were independent or not, 118 patients with PsA who had been typed for HLA class I antigens and MICA alleles were analysed for the most probable haplotypes. HLA class I and II antigens had been defined using mono or oligospecific alloantisera (180 for A, B and Cw antigens, and 120 for DR and DQ antigens) (One-Lambda, Los Angeles, CA and Fresenius, Oberursel, Germany) according to NIH standard two-stage microlymphocytotoxicity assay (11).

Most likely allele combinations were calculated using the population genetics program Arlequin (http://anthropologie.unige.ch/arlequin/) in 118 patients with PsA whose typing for both HLA class I and MICA alleles was known.

Results

The frequencies of MICA alleles in PsA patients and healthy controls are listed in Table I.

The 226 patients were subdivided for clinical subsets as previously described. A significant positive association was found between MICA-A9 (present in the 47.7 % of all PsA cases) and the PA form only when combined with SP (p_{corr}=0.0075; OR=2.82; 95% CI= 1.49-5.33) (Table I).

This PA/SP clinical subset was also characterised by a trend of lower frequency of the MICA-A5, which is however low in other clinical forms as well. Interestingly, genotype analysis reported in Table II confirmed a significant negative association of MICA-A5 when combined with MICA-A4 (p_{corr}=0.05) whereas all the other combinations involving MICA-A5 did not show any significant variation. Isolated PA, and OA with and without spondylitis did not show any significant deviation in the MICA transmembrane allele frequencies.

Moreover, the PA form among the MICA-A9 positive patients is more frequent in homozygous than in heterozygous individuals (p=0.028; OR=3.5; 95% CI=1.14-11.18) (Fig. 1).

Haplotype analysis

To verify whether the associations reported so far were independent or not,

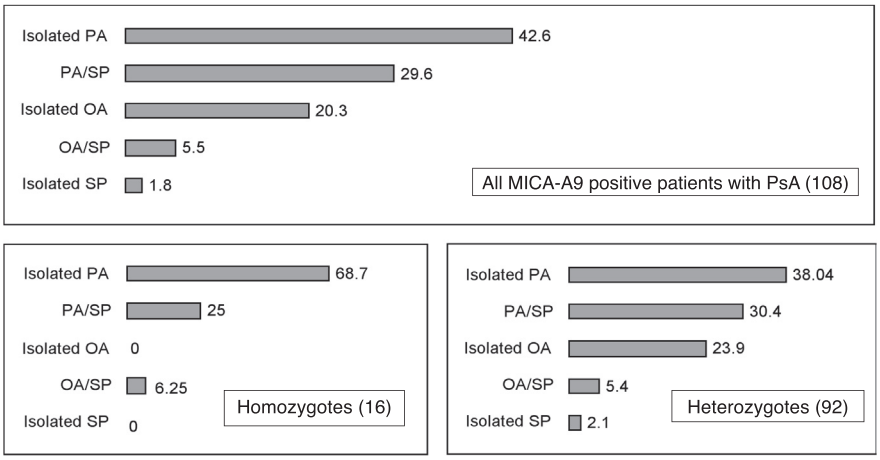

Fig. 1. Percent distribution of PsA clinical forms according to MICA-A9 zygosity.

Table III. Association of MICA-A9 with HLA-C alleles in patients with PsA.

	Patients with PsA (n=118)	
HLA-C allele	% of MICA-A9 positive (n=39)	% of MICA-A9 negative (n=79)
Cw1	0 (0)	4.5 (4)
Cw2	5.5 (2)	8.7 (7)
Cw3	3.0 (1)	4.2 (3)
Cw4	31.8 (12)	21.6 (17)
Cw5	0 (0)	10.4 (8)
Cw6	23.9 (9)	12.2 (9)
Cw7	15.2 (6)	31.8 (25)
Cw8	3.9 (2)	0 (0)
Cw12	10 (4)	2.1 (2)
others	6.7 (3)	4.5 (4)

Table IV. HLA-B; HLA-C most probable haplotypes in 39 MICA-A9 positive patients with PsA (33%) on 118 analysed.

	MICA-A9 positive patients with PsA		
HLA-B allele	%	HLA-B; C haplotype	%
B17	7.6	B17; C6	3.9
B35	29.4	B35; C2	5.2
		B35; C4	17.9
B38	28.2	B38; C4	3.9
		B38; C6	9.1
		B38; C7	5.2
B39	10.3	B39; C7	3.9
B53	5.2	B53; C4	5.2
B57	7.6	**	
B58	5.2	**	
Others	7.9		

**less than 3%

118 patients with PsA who had been typed for HLA class I antigens and MICA alleles were analysed for the most probable haplotypes. Table III reports the frequency of the HLA-C alleles in these patients who have been subdivided as MICA-A9 positive and MICA-A9 negative. An increase of the HLA-Cw4 and Cw6 and a decrease of HLA-Cw7 characterizes the cohort of the MICA-A9 positive subset; moreover, it is evident that, among the thirty-nine patients positive for MICA-A9 (33%), only 23.9% is also Cw6 positive (7.9% of the entire cohort analysed), suggesting that the two markers positively associated with the disease are not in the same haplotype. This is even clearer when the analysis is extended to the B locus (Table IV): the HLA-B alleles in linkage disequilibrium with MICA-A9 are not co-inheredited with Cw6 but rather co-segregate with other HLA-Cw alleles. Furthermore, MICA-A9 associates with several HLA-B alleles; in this regard it is interesting that about one third of the MICA-9 positive patients is also positive for HLA-B38 (Table IV). However, in this series of patients with PsA, the overall frequency of the HLA-B38 is 9.3% and the great majority of them is MICA-A9 positive as well.

Discussion

PsA is a complex disease whose pattern of clinical expressions is varied and likely to be genetically heterogeneous. Accordingly, any association study should be performed considering this possibility. As a consequence, multicenter studies, in which a high number of patients with different genetic background is available together with a classification of clinical forms, are preferred.

We analysed a microsatellite within the MICA gene, which had been reported to be associated with PsA in other populations (4), in 226 patients with PsA subdivided in clinical subsets. The results of our investigation show a strong association between MICA-A9 allele and the PA/SP clinical form of PsA.

The polymorphic MICA gene maps within the HLA region, near to the B locus and in strong linkage disequilibrium with it. The MICA gene encodes stress inducible glycoproteins tipically expressed on the membrane of intestinal epithelial cells and appears to act as ligand for NKG2D receptors expressed by NK cells, CD8+ T cells and γ/δ T lymphocyte subset, but it does not seem to be involved in antigen presentation (11, 12). MICA-A9 allele is reported to be more frequent among patients affected by type I diabetes (13) and familial Mediterranean fever (14). Moreover, the presence of a MICA allele has been reported to modulate the development of coeliac disease by conferring an additive effect in patients with concomitant genetic risk (15). Moreover, according to recently published data which show that different MICA-TM alleles associate with distinct disease forms of ulcerative colitis (16), it is conceivable that they may play a role in modulating inflammation thus influencing disease progression or topography of lesions.

MICA alleles have been found to be aberrantly expressed on synoviocytes of affected joints of patients with RA (17) and NKG2D blockade has been reported to prevent autoimmune diabetes in NOD mice (18). These data show that NKG2D plays a key role in immune mediated diseases. In this context, different MICA allele products may have different affinity for NKG2D receptor. MICA-A9 in particular is associated with greater affinity for the receptor (19). In PsA, MICA-A9 has been reported to be increased in patients with PsA independently from Cw6, so that their combined presence leads to an additional risk (4, 20).

Moreover, we found that in MICA-A9 positive patients an over-representation of the PA PsA form does exist, either

isolated or in combination with axial involvement: 15 out of 16 patients homozygous for MICA-A9 belong to the PA or PA/SP subset (Table II) suggesting a gene dosage gradient in the susceptibility to PA-PsA.

Furthermore, we report the ocurrence of a trend of a lower frequency of MICA-A5 allele in the PA clinical form of PsA. This finding prompted us to look for a phenotype not including MICA-A9. Among the possible allele combinations involving the A5 allele, only the genotype MICA-A4/A5 is significantly decreased in the PsA patients. This novel observation highlights a profile of association between MICA gene and PsA more complex than that previously described by Gonzalez *et al*. (4).

The recent observation on the possible existence of a co-operation of HLA or HLA-related genes harbored in the same haplotype in determining the susceptibility or not to ankylosing spondylitis (21) suggests that in other SpAs a similar additive effect of genes may play a role.

PsA includes distinct clinical forms characterised by different topography of joint lesions and anatomical involvement, and there are several evidence of preferential or selective associations of the clinical subsets with a distinct genetic background (HLA or HLA-related genes). In fact the presence or absence of spondylitis appears to be the most relevant discriminating feature for the genetic profile evaluated. Therefore, hunting for genes conferring susceptibility to PsA, implies a first dissection of the different clinical forms that characterise this complex disease.

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