HLA-B*27/HLA-B*07 in combination with D6S273-134 allele is associated with increased susceptibility to juvenile spondyloarthropathies

M. Harjaček[1], T. Margetić[1], V. Kerhin-Brkljačić[2], N. Martinez[2], Z. Grubić[2]

[1]Division of Pediatric Rheumatology/Immunology, Children's Hospital Zagreb;
[2]Tissue Typing Centre, University Hospital Centre Zagreb, Croatia.

Abstract

Objective

Juvenile spondylarthropathies (jSpA) are polygenic and the clustering of disease in families is caused mainly by genetic factors. Our aim was to look for possible associations of other HLA-A and B specificities, MICA and D6S273 microsatellite polymorphisms that might play a role in determining the susceptibility to jSpA.

Patients and methods

jSpA were diagnosed in 74 Croatian children, and 169 healthy unrelated individuals served as the control group. HLA class I (A, B) typing of all individuals was performed, and HLA-B7 and HLA-B27 positive subjects were subtyped by PCR-SSP method. MICA and D6S273 microsatellites alleles were analyzed by electrophoresis in an automated sequencer.

Results

*We identified 26 HLA-B*07 and 31 HLA-B*27 positive patients with jSpA. DNA subtyping of HLA-B*27 specificity demonstrated only two subtypes, B*2702 (19.35%) and B*2705 (80.65%), among jSpA patients. Subtyping analysis of HLA-B*07 gene showed presence of only one subtype, B*0702. The OR for HLA-B*07 was 2.61, while the highest OR for a single HLA specificity was found for HLA-B*27 (OR=5.60). The HLA-B*07/B*27 combination found in six children showed higher risk (OR=14.82), but the combination of specificities: HLA-B*07/HLA-B*27, and D6S273-134 allele demonstrated the highest risk (OR=26.83). The association with D6S273-134 allele was not a result of the linkage disequilibrium with HLA-B*27 specificity (LD=-0.5).*

Conclusion

*Our findings provide evidence that HLA-B*27/HLA-B*07 in combination with D6S273-134 allele is associated with increased susceptibility to jSpA in Croatian children.*

Key words

Juvenile spondyloarthropathies, HLA-B*27/B*07, D6S273-134 microsatellite, Croatian children.

Miroslav Harjaček, MD, PhD; Tanja Margetić, MD; Vesna Kerhin-Brkljačić, PhD; Natalija Martinez, PhD; Zorana Grubić, PhD.

Please address correspondence to: Dr. Miroslav Harjaček, Ass. Prof. of Pediatrics, Medical School Zagreb, Department of Pediatrics, Division of Pediatric Rheumatology/Immunology, Children's Hospital Zagreb, Klaiceva 16, 10 000 Zagreb, Croatia.
E-mail: miroslav.harjacek@zg.htnet.hr

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Introduction

The juvenile spondylarthropathies (jSpA) constitute a heterogeneous group of disorders with common genetic and clinical characteristics (1). Juvenile spondylarthropathies are polygenic in humans, but HLA-B27 is the major genetic determinant found in up to 20% of the patients (2, 3). More recently, a similar role for HLA-B7 antigen that shares some common epitopes with HLA-B27 has been proposed (4-8). Both antigens display significant levels of polymorphism, and to date, more than 40 HLA-B27 and more than 50 HLA-B7 alleles have been identified and characterized (9). It is well known that some HLA-B27 subtypes are positively (*i.e., B*2705*) and negatively (*i.e., B*2706*) associated with spondyloarthropathies (10, 11). The HLA-B*07 specificity is present in 9.67%, and the HLA-B*27 specificity allele present in 6.00% of the general Croatian population, respectively (12). In the Croatian adult population, HLA-B*2702 and B*2705 were found to be associated with ankylosing spondylitis (13). At the same time, a pilot study in Croatian children with jSpA showed clear association with B*2702 and B*2705 subtypes (14). The existence of the association between different microsatellites and diseases in various populations is well documented (15).

An MHC class I chain related A (MICA) genes, TNFB gene, as well as some microsatellites such as TNF-α D6S265 and D6S273, have drawn significant attention in recent years (16, 17). The triplet repeat (GCT) polymorphism in exon 5 of the MICA gene was found to be associated with different autoimmune diseases including spondyloarthropathies (18-20). Regions outside the MHC, particularly on chromosomes 9q31-34, 6q, 16q and 11q, have been implicated in SpA susceptibility (21-26). The LOD score for the D6S273 marker (dinucleotide microsatellite with 8 different alleles), which lies approximately 350kb centromeric to HLA-B gene in British patients, was 3.8 (27). Some D6S273 alleles appear to play a role in the susceptibility of Addison's disease, type 1 diabetes as well as in rheumatoid arthritis and Behçet's disease (28-31).

Therefore, in the present work, we have investigated the possible association of HLA-B*27, HLA-B*07 and MHC microsatellites (MICA and D6S273) in pediatric patients with jSpA and healthy controls. To our knowledge, no information is currently available on the potential role of HLA-B*07, MICA and D6S273 microsatellite polymorphisms in jSpA.

Patients and methods

We included 74 consecutive Croatian children with juvenile onset SpA seen in a period of six years (2000-2005) in our Pediatric Rheumatology Department at Children's Hospital Zagreb. Forty-five patients had undifferentiated SpA, twenty-seven had reactive arthritis, and two patients had AS. Juvenile spondiloarthropathies were diagnosed based on ESSG criteria (32). Forty-five patients with undifferentiated SpA and two patients with AS also fulfilled ILAR criteria for enthesitis related arthritis (33, 34). There were no cases with psoriatic arthritis or inflammatory bowel disease in the study group. The Children's Hospital Zagreb ethics committee approved the study. All patients signed their informed consent.

One hundred and sixty-nine healthy, unrelated individuals were selected from the general population as the control group. HLA class I (A, B) typing of all individuals was performed according to the standard microlymphocytotoxicity technique, using peripheral blood lymphocytes. Genomic DNA was also extracted from peripheral blood samples following the standard salting out protocol (35). HLA-B7 and HLA-B27 positive subjects were further subtyped by Polymerase Chain Reaction and Sequence Specific Primers (PCR-SSP) method (36, 37). The oligonucleotide primers were designed to obtain amplification of specific alleles or groups of alleles (11 HLA-B*07 alleles and 16 HLA-B*27 alleles). MICA microsatellites was amplified as suggested by Mizuki (31), while amplification conditions for D6S273 microsatellite were as suggested in protocols of 13th International Histocompatibility Workshop (38). Briefly, the 5' primer used in the amplification of each microsatellite

was end labeled with Cy5, after amplification, samples were analyzed on 6% polyacrylamide gel in an automated sequencer, ALFexpress (Pharmacia Biotech, Uppsala, Sweden). The size of the amplified fragments was determined using Fragment Manager program (Pharmacia Biotech, Uppsala, Sweden). Control DNA extracted from the 10thIHW cell lines of known microsatellite genotypes, as allelic leader, was included on every gel for standardization.

HLA allele and microsatellite allele frequencies were estimated by direct counting. Comparison of the HLA and microsatellite frequencies between the patients and control group was done using Chi-square method or the Fisher's exact test when appropriate; the *p*-value was corrected by the number of alleles observed on each genetic marker (p_c). The *p*-value was corrected for 23 of HLA-B locus, 5 of MICA locus, and 7 of D6S273 locus. The magnitude of the association was assessed by odds ratio (OR) and etiological fractions (EF) statistics (39, 40). The linkage disequilibrium (LD) was calculated by method suggested by Imanishi *et al*. (41).

Table I. Demographic characteristics of the study patients with jSpA.

No. of patients	74
Male/Female	46/28
Age at onset -mean (range) years	11.6 (3-18)
Disease duration- mean (range) months	29 (2-134)
Form of disease[#]	
Undifferentiated jSpA	45
jAS	2
ReA	27
Positive family history[*]	25

[#]Forty-seven patients (45 with undifferentiated jSpA, and 2 with jAS) fulfilled ILAR criteria for the ErA-enthesitis-related arthritis (34, 35); jAS: juvenile ankylosing spondylitis; ReA: reactive arthritis; [*]: history of rheumatic disease in the first degree relative

Table II. Pattern of joint involvement of the 74 study patients.

Patients	Hips no.	Knees no.	Enth. no.	Subt. J. no.	Si. J. no.
B*0702 (n=20)	10	3	5	2	5
B*2705 (n=21); B*2702 (n=4)	17	9	8	8	4
B*07 and B*27 negative (n=23)	7	5	9	2	6
B*07/B*27 (n=6)	3	0	2	2	2
B*27/MICA-A4/D6S273-134 (n=13)[a]	5	3	5	2	4
B*07/B*27/MICA-A4/D6S273-134 (n=5)	3	0	0	2	2
B*0702/MICA-A4/D6S273-134 (n=5)[b]	2	0	0	2	2
B*0702/MICA-A5.1/D6S273-134 (n=16)[c]	7	2	3	1	5

Enth: enthesitis; Subt. J: subtalar joints; Si. J; sacroiliac joints; no.: number of occurrences; [a]: one patient was lost to follow-up; [b]: two patients were lost to follow-up; [c]: one patient was lost to follow-up and two patients in this group had jAS with typical clinical features.

Results

Patient characteristics

Clinical features of the 74 study patients including sex, age at onset, mean duration of the disease, and positive family history of rheumatic disease in the first-degree relative are presented in Table I.

We identified 26 HLA-B*07 positive patients with jSpA, including 14 undifferentiated SpA patients, 11 ReA and one case of jAS, respectively. There were 11 girls and 15 boys; the mean age at onset of jSpA was 11.8 years (range 3.5-17.5 yrs), and the mean duration of disease was 15.3 months (range 2-64 mo). Most patients (10/26) had hip involvement Table II.

We also found 31 HLA-B*27 positive children with jSpA, among them 21 with undifferentiated SpA and 10 with ReA. There were 11 girls and 20 boys; the mean age at onset of jSpA was 12.9 years (range 5-18 yrs), and mean duration of disease was 69.9 months (range 2-134 mo). The pattern of arthritis in those children was similar to the pattern in the HLA-B7 positive group, the majority of patients (17/31) having hip involvement, whereas enthesitis was the predominant clinical feature in 9/17 children negative for both HLA-B*27 and HLA-B*07. The HLA-B*07 and -B*27 negative group of patients consisted of 6 girls and 11 boys; their mean age at onset of jSpA being 11.3 years (range 3 -17.2 yrs), and mean duration of disease 16.3 months (range 2-66 mo). Undifferentiated SpA was diagnosed in ten patients, ReA in six, and jAS in one patient, respectively. Six patients (2 female and 4 male) were both HLA-B7 and B27 positive, their average age at onset being 10.2 years (range 7-14 yrs) and mean duration of disease 14.6 months (range 3-36 mo). In three patients the final diagnosis was undifferentiated SpA and in the remaining three it was ReA.

*HLA-B*27 and HLA–B*07 association studies*

In good agreement with an earlier report on the association study in jSpA in the Croatian population HLA-B*27 and -B*07 specificities showed increase in frequencies (14), which was statistically significant, but for the HLA-B*0702 allele only before correction (Table III).

In the same group of patients we found six patients with the combination HLA-B*07/B*27 while only one healthy subject showed this combination (8.11% *vs*. 0.06%, *p*=0.0036). DNA subtyping of HLA-B*27 specificity demonstrated only two subtypes, B*2702 (19.35%) and B*2705 (80.65%), among jSpA patients. In the control group three different HLA-B*27 subtypes were found (B*2702-27.78%, B*2704-11.11%, B*2705-61.11%, respectively). Subtyping analysis of HLA-B*07 specificity showed presence of only one subtype, B*0702, in both tested groups.

Microsatelite association studies

The study of MICA and D6S273 microsatellite alleles in our group of patients demonstrated the presence of

Table III. Selected allele frequencies in patients with jSpA and controls.

Marker	Significat allele	jSpA n=74** (%)	Controls n=169 (%)	*p*	p_c	OR* (EF#)
HLA-B	*27	31 (0.4189)	19 (0.1124)	<0.00001	0.0002	5.69 (0.42)
	*0702	26 (0.3514)	29 (0.1716)	0.00356	0.0712	2.61 (0.35)
MICA	A4	43 (0.5811)	55 (0.3254)	0.00032	0.0016	2.88 (0.58)
D6S273	134	57 (0.7703)	94 (0.5562)	0.00251	0.01757	2.68 (0.77)

*OR: odds ratio; # EF: etiological fraction; **in the same group of patients we found six patients with the combination HLA-B*07/B*27, while only one healthy subject showed this combination (8.11% *vs.* 0.06%, p=0.0036; OR=14.82).

Table IV. Genotype frequencies of MICA-A4 and D6S273 alleles in HLA-B*27 positive and -B*27 negative subgroups of jSpA patients and matched controls.

Microsatellite allele	B*27 positive		B*27 negative	
	patients n=31	controls n=19	patients n=43	controls n=150
MICA-A4	31 (100%)	17 (89.47%)	12 (27.91%)	40 (26.67%)
D6S273-134	19 (61.29%)	7 (36.84%)	30 (69.77%)#	70 (46.67%)

#p=0.0124

Table V. The odds ratio (OR) of jSpA patients conferred by selected alleles alone or in combination.

Marker	OR	CI (95%)
B*07	2.61	1.40 – 4.87
B*27	5.69	2.93 – 11.06
D6S273-134	2.68	1.37 – 4.33
B*07; D6S273-134	2.72	1.34 – 5.53
B*27; D6S273-134	8.57	3.44 – 21.38
B*07/B*27	14.82	1.75 – 125.45
B*07/ B*27; D6S273-134	26.83	N/A*

OR: odds ratio: CI: confidence interval; *Not calculated due to the zero cell in table.

five and seven alleles, respectively. There were two microsatellite alleles (MICA-A4 and D6S273-134) showing higher frequency among patients with jSpA, and two microsatellite alleles (MICA-A9 and D6S273-130) showing lower frequency among patients with jSpA. However, only MICA-A4 and D6S273-134 alleles showed statistically significant association (p_c=0.00205 and p_c=0.02, respectively) with jSpA after correction of the p value. To test weather the microsatellite associations were independent of HLA-B*27 specificity or were merely a result of linkage disequilibrium (LD) between the marker alleles and HLA-B*27 specificity, we next performed stratification analysis. For that reason, we divided the subjects into B*27 positive and B*27 negative subgroups, and compared frequencies of these two microsatellite alleles in both groups. As listed in Table IV, there was no significant difference in the presence of the MICA-A4 allele between jSpA patients and controls in neither B*27 positive nor B*27 negative groups, respectively.

On the contrary, there was a significantly higher presence of MICA-A4 allele among B*27 positive subjects compared to B*27 negative subjects (p<0.00001). Applying the same analysis model, a marginally significant increase in D6S273-134 allele frequency was detected in B*27 positive patients compared to matched controls, whereas a statistically significant increase was found (p=0.0124) among B*27 negative patients, compared to matched controls. We found that MICA-A4 allele is in linkage disequilibrium with HLA-B*27 specificity (LD=2.9), however no linkage disequilibrium between HLA-B*27 specificity and D6S273-134 allele was observed (LD=-0.5).

Odds ratio (OR) analysis

The calculated odds ratio (OR) for each allele and alleles in all possible combinations is presented in Table V.

The highest OR for a single allele was found for HLA-B*27 (OR=5.60). A higher risk (OR=8.57) was observed for the combination of HLA-B*27 with D6S273-134 allele. The odds ratio was then calculated and compared, when either HLA-B*27 or D6S273-134 allele was removed from the patients and controls, respectively. When HLA-B*27 was removed, the risk of D6S273-134 allele decreased from 2.68 to 1.98 (p=0.077). In the situation when D6S273-134 allele was removed, there was also a slight decrease in the odds ratio from 5.69 to 5.47 for HLA-B*27 specificity, but it still remained significant (p=0.0008). Nevertheless, of all combinations, the HLA-B combination: HLA-B*07/B*27 showed the highest risk (OR=14.82). Finally, the HLA-B*07/HLA-B*27 in combination with D6S273-134 allele demonstrated the highest risk (OR=26.83).

Haplotype analysis

We also analyzed the HLA-A, -B haplotypic association to elucidate the potential role of HLA-A antigens in susceptibility to jSpA in the Croatian population. In the majority of HLA-B*27 positive subjects (patients and controls), HLA-B*27 was found in combination with HLA-A2 antigen. Further analysis demonstrated that among controls the haplotypic combination HLA-A3, -B*07 are significantly more common than among jSpA patients (17.9% *vs.* 6.7%, p=0.0108). The two tested groups did not show differences in distribution of HLA-A2, -B*07 haplotypic association (11.6% *vs.* 14.4%, p>0.05). Among our patients we also observed a higher frequency of HLA-A9, -B*07 haplotypic association than in controls, but without statistical significance (6.7% *vs.* 3.6%, p>0.05).

Discussion

The data reported in herein have several implications. One of the major findings of this study is the strong association of HLA-B*2705, HLA-B*0702 and D6S273-134 alleles with an increased risk of developing jSpA in the Croatian children. To our knowledge, the current study represents the first attempt to compare HLA-B specificities with MICA and D6S273 microsatellites in children with jSpA.

The contribution of the HLA region to this odds ratio depends on the model of interaction between the HLA-linked and HLA-unlinked loci. The HLA class I molecules can be classified into supertypes associated with overlapping peptide-binding motifs and repertoires. Within the HLA-B locus more than 500 alleles were identified, therefore the number of possible combinations is exceeding 35,000 (42).

While the risk for specific HLA allele in spondyloarthropaties may vary from one population group to another, the association of HLA-B27, and SpA has been well known for over 30 years. More recent studies indicate that HLA-B27 accounts for 40% of the total disease risk for AS with other MHC genes contribution of additional 10% (22). The association between HLA-B7 and SpA, antigen that share several structural similarities with HLA-B27 antigen, is less clear. It is of interest that the HLA-B*0702 allele (the only subtype found) is present in 9.67%, and the HLA-B*2705 allele (the most common one) is present in 3.55% of the general Croatian population, respectively (12, 13). The region of amino acid positions 63-71 in HLA-B27 appears to participate in the formation of at least three distinct epitopes shared by HLA-B27 and HLA-B7 identified as ME1, GSP5.3 and GS145.2, respectively (43). Others and we have found previously an increased occurrence of HLA-B7 antigen in both children and adults with SpA in various populations (6-8, 14, 44-46), while others did not (47, 48). In our study we found a significant risk of HLA-B*0702 (OR=2.61) for developing jSpA (in 20 patients), that was even more emphasized in combination with HLA-B*27 specificity (OR=14.82) found in 6 patients (Table V). Prominent clinical features in those 24 patients included hip involvement, presence of enthesitis and sacroiliitis, all very typical clinical features of jSpA. Similar findings were noted in adult patients in France (42). None of the study patients were either HLA-B27 or HLA-B7 homozygous.

In the present study, the statistically significant higher frequency of the MICA-A4 allele in the patient group was also found to be a result of linkage disequilibrium between MICA and HLA-B*27 specificity, as shown previously in the adult Croatian population, as well as in other populations (49, 50). Therefore, our data suggest that the MICA gene does not play a major or accessory role in the susceptibility to jSpA in Croatian children. On the base of collected results it is not possible to make any definitive conclusion about susceptibility/protection of HLA-A3, -B*27 haplotypic associations for jSpA. It would be useful to enlarge our group of patients as well as obtain data from other populations referring to this matter.

On the other hand, the association with D6S273-134 allele was clearly not a result of the linkage disequilibrium with HLA-B*27 specificity. The D6S273 microsatellite locus, located in the HSP70β2 region close to the TNF-α and TNF-β genes, BAT2 and mismatch repair gene MSH5, has a dinucleotide repeat motif (CA) with allele size ranging from 128bp to 142bp (51). Polymorphism at the level of D6S273 microsatellite loci was previously studied in the Croatian population. That study confirmed the irregularity in distribution of microsatellite alleles in different populations with the predominance of two or three alleles on these two investigated microsatellite loci (52). In addition, it was found that the D6S273-138 allele provides an additional risk in RA susceptibility, as well as IDDM (24, 25). Previous disease associated studies reported an association between the D6S273-128 allele and rheumatoid arthritis in Singaporean Chinese, while a study on D6S273 polymorphism in patients with Addison's disease demonstrated a relationship with the D6S273-140 allele (29, 30). In our study odds ratio for the D6S273-134 allele was 2.68, while odds ratio for the combination of B*07/B*27; D6S273-134 was 26.83. Linkage analysis performed in our population demonstrated association between B*07 and D6S273-134pb (LD=1.7) but without statistical significance. The genotype frequency of D6S273-134pb allele was quite similar among B*0702 positive patients (73.1%) as well as among B*0702 negative patients (65.2%). In contrast to the MICA-A4 allele we found no linkage disequilibrium between HLA-B*27 specificity and D6S273-134 allele (LD=-0.5). These findings might imply that a gene surrounding this location is causally related to jSpA. The HLA class III region carries many genes with immune response-related functions, and the presence of polymorphism in these genes raises the possibility of genetic liability conferred by the entire cluster (haplotype) instead of a single susceptibility gene. This possibility was already reported in Crohn's disease (53). Furthermore, D6S273 microsatellite was found to be a part of the minihaplotype in RA patients who were responders to anti-TNF-α therapy, suggesting that the D6S273 microsatellite allele may be part of a larger haplotype that may affect response to therapy (54). No data on the possible role of D6S273 polymorphism and response to therapy in jSpA patients is currently available. For that purpose, in the future studies it would be helpful to include more patients for elucidation of the role of D6S273-134bp allele in susceptibility to disease.

In conclusion, our findings support thesis about the possible presence of unidentified gene(s) around HLA-B locus which could be involved in the susceptibility to jSpA, independently of HLA-B*27 specificity. Further studies are necessary for comparison of our findings, as well as important in illumination of the role of D6S273 microsatellite in association with jSpA.

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