EXTENDED REPORT

Effect of HLA-B and HLA-DR genes on susceptibility to and severity of spondyloarthropathies in Mexican patients

G Vargas-Alarcón, J D Londoño, G Hernández-Pacheco, C Pacheco-Tena, E Castillo, M H Cardiel, J Granados, R Burgos-Vargas

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See end of article for authors' affiliations

Correspondence to:
Dr G Vargas-Alarcón,
Department of Physiology,
Instituto Nacional de
Cardiología Ignacio
Chávez, Juan Badiano No
1, Tlalpan 14080, México
DF, México;
gvargas63@yahoo.com

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Objective: To investigate the role of HLA-B and HLA-DR genes as contributors to genetic susceptibility and clinical expression of the spondyloarthropathies (SpA) in the Mexican population.

Methods: The study included 172 patients with SpA (undifferentiated SpA 83, ankylosing spondylitis (AS) 64, and reactive arthritis 25) and 99 healthy controls. The HLA-B and HLA-DR alleles were detected by the polymerase chain reaction with sequence-specific primers technique. Patient assessment included demographic data, diagnostic categories, and disease patterns. Statistical methods included the Mantel-Haenszel χ^2 test, Fisher's exact test, and Woolf method for odds ratio (OR). Differences of continuous variables between HLA allele groups were calculated by Student's *t* test.

Results: Increased frequencies of HLA-B27 ($p < 10^{-3}$, OR=28.7), HLA-DR1 ($p < 0.045$, OR=2.77), and HLA-B15 ($p = 0.034$, $p < \text{NS}$, OR=2.04) alleles in the whole group were found. HLA-B27 strength of association (OR) was 41.4 in AS; 20.9 in undifferentiated SpA; 27.2 in reactive arthritis. HLA-DR1 and HLA-B15 were increased in undifferentiated SpA ($p < 0.045$, OR=2.98 and $p = 0.004$, $p < \text{NS}$, OR=2.75). By analysing 58 HLA-B27 negative patients it was found that HLA-B15 and HLA-DR1 associations with SpA were independent of HLA-B27; increased frequencies of HLA-B15 were found in the whole SpA group and in patients with undifferentiated SpA ($p < 0.03$, OR=3.09 and $p < 0.01$, OR=3.77) and of HLA-DR1 in the latter ($p = 0.04$, $p < \text{NS}$, OR=3.15). HLA-B27 positive patients were younger than HLA-B27 negative patients at onset ($p = 0.03$), but HLA-DR1 positive patients were older than HLA-DR1 negative patients ($p = 0.03$). Bath indices for disease activity and functioning were higher in HLA-B27 positive patients ($p = 0.006$ and $p = 0.004$ v HLA-B27 negative patients). In contrast, neither HLA-DR1 nor HLA-B15 influenced these indices.

Conclusion: Apart from HLA-B27, there is a significant association of HLA-DR1 and HLA-B15 with SpA in Mexicans which is independent of B27. HLA-B27 is associated with younger age at onset and increased disease severity and HLA-DR1 with older age at onset. The strength of HLA-B15, HLA-B27, and HLA-DR1 associations varied in different forms of SpA.

The spondyloarthropathies (SpA) are a heterogeneous group of diseases characterised by axial as well as peripheral enthesitis and arthritis and less commonly by a range of extra-articular manifestations. SpA are strongly linked to genetic factors and in some patients to infections with arthritogenic bacteria. Their presentation and clinical course seem, additionally, influenced by ethnicity, age at onset, and sex.^{1,2} The strong association between HLA-B27 and the SpA, particularly ankylosing spondylitis (AS), in the general population and in first degree relatives of affected subjects gives this allele a significant role in disease susceptibility.^{3,4} Yet, the fact that only approximately 2% of HLA-B27 subjects develop AS^{5,6} and that a small proportion of patients with AS do not carry the B27 allele suggests that other genes within the major histocompatibility complex (MHC) participate in the disease susceptibility.

Previous studies have reported several non-B27 gene associations with AS and other SpA in various populations. Thus several groups have reported DR1,⁷ DR4,⁸ B60,⁹ and A9 (A*2402)¹⁰ associations with AS and a contradictory association with DR8.¹¹ Apart from HLA-B27,^{12,13} particularly B*2705,¹⁴ AS and other SpA in Mexicans have been associated with HLA-B39, FC31, DR8, and LMP2.^{15,17} However, owing to the small number of patients included in those studies and the tissue type techniques used in some of them, the association of such alleles with disease needs to be confirmed. Because of the evidence that MHC genes other than B27 may influence

disease susceptibility and clinical manifestations, we decided to study the role of HLA-B and HLA-DR genes in Mexicans with SpA.

SUBJECTS AND METHODS

Patients and control subjects

The study included 172 consecutive patients with SpA attending two specialised clinics over a period of 18 months. Eighty three of these patients were classified as undifferentiated SpA according to the European Spondyloarthropathy Study Group classification criteria,¹⁸ 64 had AS according to New York diagnostic criteria,¹⁹ and 25 reactive arthritis (ReA) according to Berlin's diagnostic proposal.²⁰ None of the patients, particularly those with undifferentiated SpA, had signs of underlying disorders such as intestinal bowel disease or psoriasis. Additionally, a group of 99 non-related healthy subjects with neither symptoms nor previous diagnosis of systemic disease were included in the study as the control group. All patients with SpA and the healthy controls and their two previous

Abbreviations: AF, aetiological fraction; AS, ankylosing spondylitis; BASDAI, Bath Ankylosing Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; MHC, major histocompatibility complex; OR, odds ratio; ReA, reactive arthritis; SpA, spondyloarthropathies

Table 1 Gene frequencies (gf) of HLA-B and HLA-DR alleles in Mexican patients with spondyloarthropathies and in healthy controls

Allele	SpA (n=172) No (gf)	AS (n=64) No (gf)	uSpA (n=83) No (gf)	ReA (n=25) No (gf)	Controls (n=99) No (gf)
B27	128 (0.37)* OR=28.7 AF=35.9%	59 (0.46)* OR=41.4 AF=44.9%	50 (0.30)* OR=20.9 AF=28.6%	18 (0.36)* OR=27.2 AF=34.6%	4 (0.02)
B15	41 (0.11)† OR=2.04 AF=6.0%	13 (0.10)	25 (0.15)† OR=2.75 AF=9.5%	3 (0.06)	12 (0.06)
B44	8 (0.02)*	0 (0.00)	6 (0.03)	2 (0.04)	16 (0.08)
B40	9 (0.02)*	0 (0.00)	8 (0.04)	0 (0.00)	20 (0.10)
B14	6 (0.01)* (n=173)	0 (0.00) (n=65)	0 (0.00) (n=83)	2 (0.04) (n=25)	14 (0.07) (n=99)
DR1	44 (0.12)* OR=2.77 AF=9.3%	15 (0.11)† OR=2.45 AF=6.8%	22 (0.13)* OR=2.98 AF=8.8%	7 (0.14)† OR=3.13 AF=9.5%	10 (0.05)
DR4	79 (0.22)	31 (0.23)	34 (0.20)	14 (0.28)	47 (0.23)
DR7	17 (0.04)	5 (0.03)	10 (0.06)	2 (0.04)	22 (0.11)
DR8	65 (0.18)	28 (0.21)	30 (0.18)	8 (0.16)	33 (0.16)

Only the relevant alleles are shown.

*pC<0.05; †p<0.05, pC=NS.

OR, relative risk; AF, aetiological fraction (%).

generations were born in Mexico. This study was approved by the institutional ethics and research committees and all subjects signed an informed consent.

HLA-B and HLA-DR alleles typing

Genomic DNA was extracted from whole blood containing EDTA by standard techniques for HLA typing.²¹ HLA-B and HLA-DR genotyping was performed by polymerase chain reaction with sequence specific primers (Pel-Freez, Brown Deer, Wisconsin, USA). Products were separated by electrophoresis in 2% agarose and visualised by ethidium bromide staining and UV transillumination. Automated gel reading was performed using Pel-Freez software (Clinical Systems, Brown Deer, Wisconsin, USA).

Clinical evaluations

Demographic data, definite diagnosis, and disease patterns were investigated in all patients by clinical history and physical examination. Disease activity and functional status were assessed by peripheral enthesitis and joint counts, lumbar, thoracic, and cervical spine measurements as well as the Bath Ankylosing Disease Activity Index (BASDAI)²² and the Bath Ankylosing Spondylitis Functional Index (BASFI).²³

Statistical analysis

Allele frequencies were compared by contingency table analysis using the Mantel-Haenszel χ^2 test, except where the number in any cell was <5 when Fisher's exact test was employed. p

Values were corrected according to the number of specificities tested and the number of comparisons performed. The level of significance was established at pC<0.05. The statistical program "EPIINFO" (version 5.0; USD Incorporated 1990, Stone Mountain, Georgia) was used for these analyses. The magnitude of associations was assessed using odds ratio (OR) and aetiological fraction (AF) statistics.²⁴ Confidence intervals were calculated for ORs by Woolf's method.²⁵ Student's *t* test was used to calculate the significance of the differences in continuous variables between HLA groups. The 6.1 SSPS program for Windows was used for these analyses.

RESULTS

HLA and disease susceptibility

As expected, there was an increased frequency of HLA-B27 in the whole group of patients with SpA (pC<10⁻³, OR=28.7, AF=35.9%) and, additionally, a significant increase of HLA-DR1 (pC=0.045, OR=2.77, AF=9.3%) and a moderate increase of HLA-B15 (p=0.034, pC=NS, OR=2.04, AF=6.0%). In contrast, the frequencies of HLA-B44, HLA-B40, and HLA-B14 were decreased in comparison with healthy controls (pC=0.03, pC=0.003, and pC=0.03, respectively) (table 1).

The strength of the allelic association varied between the three diagnostic categories. For the HLA-B27 allele, the strongest association was with AS (pC<10⁻³, OR=41.4, AF=44.9%) and the weakest with undifferentiated SpA (pC<10⁻³, OR=20.9, AF=28.6%). A significant increase of

Table 2 Gene frequencies (gf) of HLA-B and HLA-DR alleles in HLA-B27 negative patients with spondyloarthropathies and in healthy controls

Allele	SpA (n=58) No (gf)	AS (n=11) No (gf)	uSpA (n=37) No (gf)	ReA (n=10) No (gf)	Controls (n=95) No (gf)
B39	23 (0.19)	4 (0.18)	13 (0.17)	6 (0.30)	27 (0.14)
B15	20 (0.17)* OR=3.09 AF=11.6%	3 (0.13)	15 (0.20)* OR=3.77 AF=14.8%	2 (0.10)	12 (0.06)
B35	15 (0.12)	2 (0.09)	9 (0.12)	4 (0.20)	26 (0.13)
B51	9 (0.07)	3 (0.13)	5 (0.06)	1 (0.05)	14 (0.07)
DR1	11 (0.09)	1 (0.04)	9 (0.12)† OR=3.15 AF=8.3%	1 (0.05)	8 (0.04)

Only the relevant alleles are shown.

*pC<0.05; †p<0.05, pC=NS.

OR, relative risk; AF, aetiological fraction (%).

Table 3 Effect of HLA-B27, HLA-DR1, and HLA-B15 on age at onset and duration of the disease

	HLA-B27			HLA-DR1			HLA-B15		
	Positive	Negative	p	Positive	Negative	p	Positive	Negative	p
Number (%)	113 (67)	55 (33)		48 (28)	121 (72)		38 (23)	130 (77)	
Current age (years), mean (SD)	28.6 (9.0)	29.9 (10.0)	NS	29.2 (8.7)	29.0 (9.7)	NS	31.3 (10.9)	28.3 (8.8)	NS
Sex male/female	84/29 (74/26%)	29/26 (53/47%)	0.0069*	32/16 (67/33%)	82/39 (68/32%)	NS	24/14 (63/37%)	89/41 (68/32%)	NS
Age at onset (years), mean (SD)	20.1 (8.0)	23.1 (9.9)	0.038†	23.4 (9.2)	20.2 (8.4)	0.030†	23.0 (10.1)	20.5 (8.2)	NS
Duration of the disease (years), mean (SD)	8.4 (7.8)	6.8 (7.17)	NS	5.7 (5.3)	8.8 (8.2)	0.019†	8.3 (7.9)	7.8 (7.5)	NS

*Dichotomic variables, χ^2 ; †Continuous variable, Student's *t* test.

NS, not significant.

This analysis was made in 168 patients.

HLA-DR1 ($p=0.045$, $OR=2.98$, $AF=8.8\%$) and a moderate increase of HLA-B15 ($p=0.004$, $pC=NS$, $OR=2.75$, $AF=9.5\%$) was found in patients with undifferentiated SpA, but not in patients with AS or ReA (table 1). Patients with AS and ReA showed a moderate increase of the HLA-DR1 allele ($p=NS$, $OR=2.45$, $AF=6.8\%$ and $pC=NS$, $OR=3.13$, $AF=9.5\%$, respectively).

To establish whether HLA-DR1 and HLA-B15 associations with SpA were dependent on the presence of HLA-B27 or not, the frequencies of HLA-B and HLA-DR alleles were analysed in 58 HLA-B27 negative patients with SpA (11 with AS, 37 with undifferentiated SpA, and 10 with ReA) (table 2). This analysis confirmed an increased frequency of HLA-DR1 ($p=0.04$, $pC=NS$, $OR=3.15$, $AF=8.3\%$) and HLA-B15 ($p<0.01$, $OR=3.77$, $AF=14.8\%$) in undifferentiated SpA and of HLA-B15 ($p=0.03$, $OR=3.09$, $AF=11.6\%$) in SpA as a whole.

HLA tissue types and clinical correlation

There were significant correlations between HLA-B27, HLA-B15, and HLA-DR1 alleles with various demographic and clinical parameters in the SpA group taken as a whole (tables 3 and 4). The proportion of men in comparison with women in the group of subjects carrying HLA-B27 was higher than in HLA-B27 negative subjects. The mean age at onset in HLA-B27 patients was lower than that found in HLA-B27 negative patients (20.1 (8.0) years *v* 23.1 (9.9) years; $p=0.038$). In contrast, the mean age at onset in patients with HLA-DR1 was higher than that found in those without this allele (23.4 (9.2) years *v* 20.2 (8.4) years; $p=0.030$). Regardless of diagnosis, in patients with HLA-B27 the BASDAI ($p=0.006$) and BASFI ($p=0.004$) scores were significantly higher, and the modified Schober's test and chest expansion were lower ($p=0.02$ and $p=0.03$, respectively). However, none of these findings reached statistical significance when analyses were carried out in each of the three diagnostic categories (data not shown). Although disease duration in patients with HLA-B27 was slightly longer than that of HLA-B27 negative patients, the difference between the two groups was not statistically significant ($p=0.202$).

DISCUSSION

The results of this study suggest that besides the strong association between HLA-B27 and each of the three clinical forms included in the study, the SpA seen in the Mexican mestizo are associated with other MHC genes. Thus we found an increased prevalence of HLA-B15 and HLA-DR1 in some specific clinical subgroups. In contrast, we could not confirm the association between SpA or AS with HLA-DR8 and B39 reported in previous studies.^{15,16} Differences between these studies may be due to the number of patients studied, case selection, and tissue typing techniques. In this study the inclusion of a cohort of patients seen by a single group of investigators and the use of DNA based genotyping methods certainly reduced selection bias and the rate of false positive and negative results.

The prevalence of HLA-B15, an allele previously associated with AS and ReA,²⁶ was increased in both the whole group of patients with SpA and particularly in patients with undifferentiated SpA. Mielants *et al* found a significant increase of HLA-Bw62 (HLA-B15 split) in ReA, in undifferentiated SpA, and in HLA-B27 negative patients with AS associated with inflammatory bowel disease and, particularly, Crohn-like lesions in the colon and terminal ileum.²⁶ In our study, only 22/38 HLA-B15 positive patients were Bw62 and this split was not significantly higher in patients than in healthy controls (data not shown). Because none of our patients had clinical evidence of inflammatory bowel disease, HLA-B15 appears unrelated to gut disease in our group, particularly undifferentiated SpA. In another study,²⁷ HLA-Bw62 was associated with clinical remission, in contrast with the HLA-B27 relation with severe disease found in this study.

The strong association of SpA with the presence of HLA-B27 is well known, thus the possible association of these diseases with other genetic markers needs to take this fact into account. It was not possible to include B27 positive healthy subjects in our study owing to the low frequency of HLA-B27 in the general Mexican mestizo population. To overcome this problem and establish whether HLA-B15 and HLA-DR1 associations with SpA were dependent on the presence of

Table 4 Effect of HLA-B27, HLA-DR1, and HLA-B15 on disease activity and functioning indices

	HLA-B27			HLA-DR1			HLA-B15		
	Positive	Negative	p	Positive	Negative	p	Positive	Negative	p
Number (%)	113 (67)	55 (33)		48 (28)	121 (72)		38 (23)	130 (77)	
BASDAI, mean (SD)	4.7 (2.2)	3.6 (2.4)	0.006*	4.3 (2.4)	4.4 (2.4)	NS	4.5 (2.3)	4.3 (2.4)	NS
BASFI, mean (SD)	4.3 (2.7)	3.1 (2.3)	0.004*	3.9 (2.8)	3.9 (2.5)	NS	3.8 (2.5)	3.4 (2.6)	NS
Schober's test (cm), mean (SD)	4.0 (1.8)	4.6 (1.2)	0.02*	4.5 (1.6)	4.1 (1.7)	NS	4.5 (1.4)	4.1 (1.7)	NS
Chest expansion (cm), mean (SD)	3.6 (1.4)	4.1 (1.4)	0.03*	3.8 (1.4)	3.8 (1.4)	NS	3.86 (1.3)	3.8 (1.5)	NS

BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index.

*Continuous variable, Student's *t* test.

NS, not significant.

This analysis was made in 168 patients.

HLA-B27 or not, the frequencies of HLA alleles were analysed only in HLA-B27 negative patients and healthy controls. The increased frequency of HLA-B15 was still significant in the group of HLA-B27 negative subjects, suggesting that the influence of the B15 allele on disease susceptibility does not depend on the presence of HLA-B27. Nine of the 20 patients with B*15 in our study carried the B*1522 subtype. In contrast with HLA-B27, this B*15 subtype (and HLA-B40) has interestingly no Glu and Cys residues in positions 45 and 67.²⁸ A role for Glu⁴⁵ and Cys⁶⁷ residues on some B15 subtypes has been suggested as a possible susceptibility factor in these patients. Our data, therefore, possibly do not support the role of Glu⁴⁵ and Cys⁶⁷ residues in genetic susceptibility to SpA in the Mexican mestizo population. To test this hypothesis, we require further high resolution genotyping techniques to better identify HLA-B15 subtypes.

The HLA-DR1 class II allele was significantly associated with SpA as whole in this study. The association was also significant in the group of patients with undifferentiated SpA. Recently, the association between this allele and AS has been described in a large group of British patients.⁷ In contrast, HLA-DR1 was not significantly associated with AS in our group. Although sample size may account for this result, the group of patients with AS included in this study were young subjects with a high prevalence of peripheral enthesopathy and arthropathy. This pattern, which differs from that seen in white subjects,²⁹⁻³⁰ may account for this apparent lack of association of AS with HLA-DR1. The association of HLA-DR1 with undifferentiated SpA in a slightly older group of patients might distinguish a group of patients evolving to AS, but we have not followed up our group for more than two years.

As expected we found a significant association between SpA and HLA-B27. In AS, the frequency of this allele was higher than the figures of between 68.6 and 77.6% reported by others.¹²⁻¹³ Several features of SpA, particularly age at onset, sex, and disease severity, appeared to be influenced by HLA-B27. Thus, patients with SpA with HLA-B27 were mostly young men who had a more severe and active disease as measured by the BASDAI, BASFI as well as spinal mobility and chest expansion at the time of the study. Although disease duration did not differ among HLA-B27 positive and negative patients with SpA, its role in disease severity cannot be ruled out. HLA-B27 subjects with AS or ReA follow a severe and sometimes disabling course of disease accompanied by anterior uveitis and heart disease. In contrast, neither HLA-DR1 nor HLA-B15 influenced disease severity.

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Authors' affiliations

G Vargas-Alarcón, G Hernández-Pacheco, E Castillo, Department of Physiology, Instituto Nacional de Cardiología Ignacio Chávez, México DF, México

J D Londoño, M H Cardiel, J Granados, Department of Immunology and Rheumatology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, México DF, México

C Pacheco-Tena, R Burgos-Vargas, Department of Rheumatology, Hospital General de México, México DF, México

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