

EXTENDED REPORT

Heat shock protein 70 gene polymorphisms in Mexican patients with spondyloarthropathies

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Objective: To investigate the role of HSP70 genes as contributors to genetic susceptibility of the spondyloarthropathies (SpA) in the Mexican population.

Methods: The study included 150 patients with SpA (undifferentiated spondyloarthropathy (uSpA) 68, ankylosing spondylitis (AS) 60, and reactive arthritis 22) and 158 healthy controls. HSP70-1, HSP70-2 and HSP70-hom genotypes were analysed by the polymerase chain reaction-restriction fragment length polymorphism technique. Statistical methods included the Mantel-Haenszel, χ^2 , Fisher's exact test, and Woolf's method for odds ratio (OR).

Results: HSP70-2 B/B genotype frequency was increased in the whole group of patients with SpA ($p < 0.05$, OR=4.3), as well as in the different clinical subgroups ($p < 0.05$, OR=4.2 for AS; $p < 0.05$, OR=4.4 for uSpA; and $p < 0.05$, OR=4.1 for ReA). This frequency remained significantly increased when the patients with B27 negative SpA were analysed. On the other hand, HSP70-hom locus analysis showed significantly increased frequency of A allele in the whole group of SpA ($p < 0.05$, OR=3.4), as well as in the groups with AS ($p < 0.05$, OR=5.6) and with uSpA ($p < 0.05$, OR=3.1), when compared with healthy controls. In this case, also, the genotype A/A was increased in the whole group of SpA ($p < 0.05$, OR=4.5), as well as in patients with AS ($p < 0.05$, OR=6.4) and with uSpA ($p < 0.05$, OR=3.7). When the patients with B27 negative SpA were analysed the frequencies of HSP70-hom A allele and A/A genotype remained significantly increased in the whole group of SpA ($p < 0.05$, OR=3.2 for the A allele and $p < 0.05$, OR=4.2 for the A/A genotype) and in the uSpA subgroup ($p < 0.05$, OR=3.8 for the A allele and $p < 0.05$, OR=4.3 for the A/A genotype).

Conclusion: In addition to the association of SpA with HLA-B27, there is a significant association of HSP70-2 and HSP70-hom alleles with SpA in Mexicans. This association seems to be independent of the susceptibility conferred by HLA-B27 in the group of patients with uSpA.

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The spondyloarthropathies (SpA) constitute a group of diseases of unknown cause which share clinical, epidemiological, and genetic features.¹ The pathogenesis of SpA is attributed in part to the interaction between genetic and, in some patients, environmental factors. Their clinical expression seems additionally influenced by ethnicity, age at onset, and sex of the patients.² The role of genetic factors in the pathogenesis of SpA is best represented by the strong association between the HLA-B27 antigen and ankylosing spondylitis (AS) and then with undifferentiated spondyloarthropathy (uSpA), reactive arthritis (ReA), and the other SpA.^{3–4} None the less, on the one hand, only 1–3 % of HLA-B27 positive subjects from the population develop AS and, on the other, not all patient with SpA carry the HLA-B27 antigen, indicating the possibility that other genes may influence disease susceptibility.^{5–6}

Besides studying the role of non-HLA-B27 major histocompatibility complex alleles in the pathogenesis of SpA, few reports have considered the role of the heat shock protein 70 (HSP70) locus on disease susceptibility. The HSP70 locus is located close to HLA-B, and the polymorphism of its genes includes HSP70-2 and two other immediately adjacent genes, HSP70-1 and HSP70-hom, map-90 kb telomeric to the C2 locus and 280 kb centromeric to the tumour necrosis factor α locus.⁷ Because the polymorphism of HSP70 genes has been linked to autoimmune disease in some studies,^{8–10} its role has been investigated in AS, but no significant associations were found in Finnish and Spanish patients.^{11–12}

In this study we explored the possibility that HSP70 genes might be involved in the susceptibility to SpA in Mexican Mestizo patients, by using the polymerase chain reaction

(PCR) and restriction fragment length polymorphism analyses. Clinically, there is some evidence that SpA and particularly AS in Mexicans differ from that seen in most white populations, suggesting a role for additional factors in their pathogenesis.^{13–14}

SUBJECTS AND METHODS

Study subjects

The study included 150 consecutive patients with SpA¹⁵ attending two specialised clinics over a period of 18 months. Sixty eight of these patients had uSpA, 60 AS,¹⁶ and 22 ReA.¹⁷ The group of patients with uSpA all fulfilled the SpA classification criteria,¹⁵ but none had signs of underlying disorders such as intestinal bowel disease or psoriasis. A group of 158 non-related healthy subjects with neither symptoms nor previous diagnosis of systemic disease comprised the control group. All patients with SpA and the healthy controls and their two previous generations were born in Mexico. This study was approved by the institutional ethics and research committees and all subjects signed an informed consent form.

DNA extraction

Genomic DNA from whole blood containing EDTA was extracted by standard techniques.¹⁸

Abbreviations: AF, aetiological fraction; AS, ankylosing spondylitis; HSP, heat shock protein; OR, odds ratio; PCR, polymerase chain reaction; ReA, reactive arthritis; SpA, spondyloarthropathies; uSpA, undifferentiated spondyloarthropathy.

HSP70 polymorphism analysis**HSP70-1**

The biallelic polymorphism at position 190 in the HSP70-1 gene was detected by *BsrBI* restriction enzyme digestion of the fragment 325 bp DNA, which was previously amplified by using the following primers: 5'-TCCGGCGTCCGGAAGG ACC-3' (forward) and 5'-TGCGGCCAATCAGGCGCTT-3' (reverse).¹⁹ The presence of the 171 bp, 84 bp, and 70 bp fragments represents the HSP70-1*b1 allele, whereas the b2 allele showed two fragments of 241 bp and 84 bp.

HSP70-2

An HSP70-2 restriction fragment length polymorphism at position 1267 was characterised by a PCR procedure. This analysis was performed considering the polymorphic *PstI* site at position 1267 of these alleles. We selected the following primers spanning the polymorphic *PstI* site (nucleotide 1267) according to the previously described sequence,²⁰ sense (nucleotide 1083–1102 within the coding region) 5'-CATCGACTTCTACA CGTCCA-3' and antisense (nucleotide 2180–2199 within the 3' untranslated end to avoid HSP70-1 homology) 5'-CAAAGT CCTTGAGTCCCAAC-3'. The products of PCR were cleaved with *PstI* (Pharmacia, Uppsala, Sweden) and electrophoresed on 1.5% agarose stained with ethidium bromide. The DNA-lacking polymorphic *PstI* site within the HSP70-2 gene generates a product of 1117 bp after restriction (A allele), whereas the *PstI* site produced two fragments of 936 and 181 bp (B allele).

HSP70-hom

A fragment of 878 bp that includes the polymorphic site on position 2437 was amplified using the primers 5'-GGACAAGTCTGAGAAGGTACAG-3' (forward) and 5'-GTAAGTATTAGATTGAGTCTGG-3' (reverse) and subject to restriction analysis with *NcoI* as previously described.²⁰ In this case the presence of two fragments of 551 bp and 327 bp represents the A allele, whereas the complete fragment (878 bp) was named the B allele.

Statistical analysis

Genotype and allele frequencies were compared by contingency table analysis using the Mantel-Haenzel, χ^2 test, or the

Fisher's exact test if the number in any cell was <5. p Values were corrected according to the number of specificities tested and the number of comparisons performed. The level of significance was established at $p < 0.05$. 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 the odds ratio (OR) and aetiological fraction (AF) statistics.²¹ Confidence intervals were calculated for the OR by Woolf's method.²²

RESULTS

One hundred and fifty patients with SpA (106 men, 44 women) were included in the study, with a mean (SD) age at onset of 21.1 (9.5) years. Sex distribution and mean age at onset differed between groups: (a) uSpA (n=68): 45 men, 23 women; 22.2 (9.4) years; (b) AS (n=60): 48 men, 12 women; 17.5 (7.9) years; and (c) ReA (n=22): 13 men, 9 women; 26.2 (10.8) years.

The polymorphism of HSP70-1 was similar in all three groups of patients with SpA and in the control group (data not shown). In contrast, the allele and genotype frequencies of HSP70-2 and HSP70-hom were significantly different in the SpA group than in the healthy subjects (table 1). Thus HSP70-2 B/B genotype frequency was increased in the whole group of SpA ($p < 0.05$, OR=4.3, AF=14.3%) as well as in AS ($p < 0.05$, OR=4.2, AF=13.9%), uSpA ($p < 0.05$, OR=4.4, AF=14.7%), and ReA ($p < 0.05$, OR=4.1, AF=13.7%). For the HSP70-hom locus, there was a significant increase of the A allele in the whole group of SpA ($p < 0.05$, OR=3.4, AF=66.6%), AS ($p < 0.05$, OR=5.6, AF=78.6%), and uSpA ($p < 0.05$, OR=3.1, AF=62.4%). Additionally, the HSP70-hom A/A genotype was significantly increased in the whole group of SpA ($p < 0.05$, OR=4.5, AF=68.4%) as well as in the AS ($p < 0.05$, OR=6.4, AF=76.8%) and uSpA ($p < 0.05$, OR=3.7, AF=62.9%) groups.

To establish whether HSP70 associations with SpA were dependent on the presence of HLA-B27 or not, the frequencies of HSP70 alleles were analysed only in HLA-B27 negative patients with SpA (table 2). This analysis showed an increased frequency of HSP70-2 B/B genotype in the whole group of SpA

Table 1 HSP70-2 and HSP70-hom genotype and allele frequencies (%) in Mexican patients with SpA and healthy controls

	SpA (n=150) No (%)	AS (n=60) No (%)	uSpA (n=68) No (%)	ReA (n=22) No (%)	Controls (n=158) No (%)
HSP70-2					
Genotype					
A/A	45 (30)	16 (27)	21 (31)	8 (36)	36 (23)
A/B	77 (51)*	33 (55)*	34 (50)*	10 (45)*	114 (72)
B/B	28 (19)*	11 (18)*	13 (19)*	4 (18)*	8 (5)
	OR=4.3	OR=4.2	OR=4.4	OR=4.1	
	AF=14.3%	AF=13.9%	AF=14.7%	AF=13.7%	
Alleles					
A	167 (56)	65 (54)	76 (56)	26 (59)	186 (59)
B	133 (44)	55 (46)	60 (44)	18 (41)	130 (41)
HSP70-hom					
Genotype					
A/A	130 (88)*	51 (91)*	60 (86)*	19 (86)	97 (61)
	OR=4.5	OR=6.4	OR=3.7		
	AF=68.4%	AF=76.8%	AF=62.9%		
A/B	15 (10)*	5 (9)*	9 (13)*	1 (5)*	56 (35)
B/B	3 (2)	0 (0)	1 (1)	2 (9)	5 (3)
Alleles					
A	275 (93)*	107 (96)*	129 (92)*	39 (89)	250 (79)
	OR=3.4	OR=5.6	OR=3.1		
	AF=66.6%	AF=78.6%	AF=62.4%		
B	21 (7)*	5 (4)*	11 (8)*	5 (11)	66 (21)

* $p < 0.05$.

OR, odds ratio; AF, aetiological fraction.

($pC < 0.05$, $OR = 5.3$, $AF = 16.5\%$) and in each one of the clinical subgroups: AS ($pC < 0.05$, $OR = 5.2$, $AF = 16.1\%$), uSpA ($pC < 0.05$, $OR = 4.3$, $AF = 13.1\%$), and ReA ($pC < 0.05$, $OR = 10.5$, $AF = 30.1\%$). In contrast, the frequencies of the HSP70-hom A allele and A/A genotype were only significantly increased in the whole group of SpA ($pC < 0.05$, $OR = 3.2$, $AF = 64.1\%$ for the A allele and $pC < 0.05$, $OR = 4.2$, $AF = 66.6\%$ for the A/A genotype) and in uSpA ($pC < 0.05$, $OR = 3.8$, $AF = 69.8\%$ for the A allele and $pC < 0.05$, $OR = 4.3$, $AF = 67.3\%$ for the A/A genotype).

DISCUSSION

The results of this study showed a significant association of HSP70-2, particularly HSP70-2 B/B, with the whole group of SpA and the three different diagnostic subgroups, and a significant increase of HSP70-hom locus alleles was seen in some subgroups, suggesting a role for these genes in disease susceptibility in the Mexican Mestizo population. In contrast with findings in the Finnish and Spanish populations with AS,^{11,12} our study detected increased frequencies of the HSP70-hom A allele and A/A genotype in the whole group of SpA as well as in the subgroups of patients with AS and uSpA. The strong association of SpA with the presence of HLA-B27 antigen is well known, and thus the possible association of these diseases with other genetic markers needs to be considered. It was not possible to include B27 positive healthy subjects in our study because of the low frequency of HLA-B27 in the general Mexican Mestizo population. To avoid this problem and establish whether HSP70 associations with SpA were dependent on the presence of HLA-B27 or not, the frequencies of HSP70 alleles were analysed only in HLA-B27 negative patients and healthy controls. When we analysed these groups, the increased frequencies of the HSP70-hom A allele and A/A genotype remained significantly increased in the whole group of SpA and in the subgroup of patients with uSpA. None the less, the fact that we only had eight patients with HLA-B27 negative AS in the analysis should be taken into account when interpreting the role of HSP70-hom polymorphism in AS.

Recent papers have shown that the peptide binding domains of the HSP70 and HLA class I molecule are remarkably similar and even a structural model for the former has been developed.²³ On the other hand, the HSP70-hom polymorphic site detected by *NcoI* is located on position 2437, which corresponds to a Met→Thr amino acid substitution at position 493.²⁰ In the hypothetical model, the 493 residue is located on the β sheets, which form the floor of the peptide binding site of the molecule. In this context, it is thought that this substitution may be associated with some variation in the peptide-binding specificity of different HSP70-hom haplotypes.

Previous studies have shown an association between the HSP70-2 B/B genotype and autoimmune disease,⁸⁻¹⁰ but the mechanisms are largely unknown. Findings in rheumatoid arthritis suggest that the HLA-DR4 and DR10 motifs associated with the disease bind to a 70 kDa HSP²⁴ which perhaps could influence the processing of antigenic peptides and their inclusion in the HLA-DRB1 chain groove. This effect might be expected with the HSP70-hom variation because it takes place within the peptide binding domain predicted,²³ but possibly not with HSP70-2 because its polymorphism depends on a silent change (A→G) in the coding region.

The role of HSP70-2 in the pathogenesis of SpA nevertheless remains to be determined. Unless a quantitative difference in HSP70-2 expression between carriers of the B/B genotype and those carrying other genotypes (A/A or A/B) exists, any contribution of the HSP70-2 polymorphism to the pathogenesis of SpA might be attributed to a neighbouring, yet unidentified gene. Thus a decrease of the HSP70-2 mRNA expression in homozygotic subjects for the B allele might occur in comparison with its expression in A/B heterozygotes.²⁵ In such a case, the cell response of B/B subjects to stress would be impaired and lead to the intracellular accumulation of denaturalised protein or peptide transporting defects, affecting self tolerance.

In conclusion, our study shows that the SpA in Mexican Mestizo patients, in addition to its association with HLA-B27, is also associated with some HSP-70 alleles. This association seems to be independent of the susceptibility conferred by the HLA-B27 in the uSpA group of patients. Additional studies in

Table 2 HSP70-2 and HSP70-hom genotype and allele frequencies (%) in B27 negative Mexican patients with SpA and healthy controls

HSP70-2	SpA (n=54) No (%)	AS (n=10) No (%)	uSpA (n=35) No (%)	ReA (n=9) No (%)	Controls (n=154) No (%)
Genotype					
A/A	15 (28)	3 (30)	9 (26)	3 (33)	34 (22)
A/B	28 (52)*	5 (50)	20 (57)	3 (33)*	113 (73)
B/B	11 (20)*	2 (20)*	6 (17)*	3 (33)*	7 (5)
	OR=5.3 AF=16.5%	OR=5.2 AF=16.1%	OR=4.3 AF=13.1%	OR=10.5 AF=30.1%	
Alleles					
A	58 (54)	11 (55)	38 (54)	9 (50)	181 (59)
B	50 (46)	9 (45)	32 (46)	9 (50)	127 (41)
HSP70-hom	SpA (n=47)	AS (n=8)	uSpA (n=32)	ReA (n=7)	Controls (n=154)
Genotype					
A/A	41 (87)*	7 (88)	28 (88)*	6 (86)	95 (62)
	OR=4.2 AF=66.6%		OR=4.3 AF=67.3%		
A/B	5 (11)*	1 (13)	4 (13)*	0 (0)	54 (35)
B/B	1 (2)	0 (0)	0 (0)	1 (14)	5 (3)
Alleles					
A	87 (93)*	15 (94)	60 (94)*	12 (86)	244 (79)
	OR=3.2 AF=64.1%		OR=3.8 AF=69.8%		
B	7 (7)*	1 (6)	4 (6)*	2 (14)	64 (21)

* $pC < 0.05$.

OR, odds ratio; AF, aetiological fraction.

a large number of patients and the inclusion of B27 positive healthy subjects might help to establish the true significance of these associations in the Mexican population.

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