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ORIGINAL ARTICLE

*HLA-B*27* — Frequency of clinical signs in Brazilian patients with spondyloarthritis

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Abstract Spondyloarthritis presents clinical features, laboratory findings, and similar images, but their clinical manifestations reveal great heterogeneity in patients *HLA-B*27* positive and negative. This study compared the frequencies of the clinical manifestations in the presence and absence of *HLA-B*27*. From the 156 patients with clinical suspicion of spondyloarthritis, 73 had a diagnosis of spondyloarthritis confirmed. The *HLA-B*27* gene was identified by polymerase chain-reaction sequence-specific oligonucleotide probe (PCR-SSOP). The Student *t* test was used to calculate the values of mean and the Fisher's exact test was used to compare proportions. The values of odds ratio (OR) and confidence interval (CI) at 95% were also calculated ($p < 0.05$). The spondyloarthritis found were: ankylosing spondylitis ($n = 47$, 64.4%), psoriatic spondyloarthritis ($n = 9$, 12.3%), undifferentiated spondyloarthritis

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(*n* = 9, 12.3%), enteropathic spondyloarthritis (*n* = 6; 8.2%) and reactive spondyloarthritis (*n* = 2, 2.7%). Overall, 35 (47.9%) patients were *HLA-B*27* positive and 38 (52.1%) were negative. This gene was associated with ankylosing spondylitis (OR: 5.37, 95% CI: 1.813—15.905, *p* = 0.003) but not with enteropathic spondyloarthritis (OR: 0.07, 95% CI: 0.003—1.301, *p* = 0.025). The sacroiliitis was associated with *HLA-B*27* positive (OR: 10.552, 95% CI: 1.260—88.256, *p* = 0.014) and intestinal injury with *HLA-B*27* negative (OR: 0.195, 95% CI: 0.038—0.978, *p* = 0.048). The image signals sacroiliitis were associated with the *HLA-B*27* gene while intestinal involvement was not associated with this gene.

Introduction

Spondyloarthritis is a group of diseases with common clinical, laboratory, and image findings. In this group fall ankylosing spondylitis, psoriatic spondyloarthritis, enteropathic spondyloarthritis (spondyloarthritis associated with Crohn's disease or ulcerative colitis), reactive spondyloarthritis, and undifferentiated spondyloarthritis.[1] These diseases are characterized by frequent inflammatory joint involvement of the sacroiliac joints (sacroiliitis) and/or peripheral joints, predominantly oligoarthritis of large joints of the lower limbs. Ocular inflammatory lesions (uveitis), bowel (colitis nonspecific), or enthesitis (heel enthesitis) are additional findings in spondyloarthritis. Specific manifestations for each of the diseases belonging to the spondyloarthritis group are also frequently reported.[1] Furthermore, spondyloarthritis is commonly associated with the *HLA-B*27* gene, but the prevalence of this gene varies with each specific disease.[1—3]

Different studies have analyzed the clinical manifestations of spondyloarthritis in patients with and without *HLA-B*27*, but the results revealed great heterogeneity. In a previous study, it was observed that the presence of this gene in patients with back pain increases the risk of sacroiliitis by 50%.[4] However, assessing the involvement of the hip joint in patients with spondyloarthritis, Burki and colleagues[5] did not find association between this involvement and the presence of *HLA-B*27*. Also, another study reported no association between this gene and acute anterior uveitis in patients with spondyloarthritis.[6] However, in another recent study, the authors reported that the occurrence of uveitis in patients with spondyloarthritis is an event common in patients carrying the *HLA-B*27* gene.[7] These data demonstrate the disagreement in the literature on the relationship between the clinical manifestations of spondyloarthritis and the *HLA-B*27* gene. The aim of this study was to compare the frequencies of the main clinical signs of spondyloarthritis in the presence and absence of the *HLA-B*27* gene.

Materials and methods

Ethical consideration

This cross-sectional study was approved by the Research Ethics Committee of Medical School of São José do Rio Preto (FAMERP), São José do Rio Preto (Case 055/2011) and each participant, after receiving all the information about the study objectives as well as on the procedures performed, signed the consent form.

Composition of the study groups

From June 2007 to May 2010, 156 patients with clinical suspicion of spondyloarthritis were referred to the Outpatient Clinic of Rheumatology from the Regional Medical Faculty Foundation (FUNFARME). All of them were investigated for the presence or absence of the *HLA-B*27* gene at the Immunogenetics Laboratory of the Molecular Biology Department from Medical School of São José do Rio Preto (FAMERP). Overall, 73 patients had a clinical diagnosis of spondyloarthritis confirmed according to the criteria of the European Study Group Spondyloarthropathy (ESSG) used at the time.[8] Clinical information was obtained from the medical records of patients.

Blood sampling and genomic DNA extraction

A sample of 5 mL of peripheral blood was collected in tubes with EDTA from each individual by venipuncture. The genomic DNA was extracted from the white blood cells using a commercial kit (PureLink, Invitrogen®, Carlsbad, CA, USA).

*HLA-B*27* genotyping

The *HLA-B*27* genotyping was performed by polymerase chain reaction-specific sequence oligonucleotide (PCR-SSO) in a low resolution system (One Lambda INC, Canoga Park, CA, USA) with Luminex technology. The manufacturer's recommendations were strictly followed.

Statistical analysis

The data were compared using Fisher's exact test (GraphPad Instat version 3.06, GraphPad Software, Inc., La Jolla, CA, USA). The mean values for age were calculated by the Student *t* test. Odds ratio (OR) and confidence interval (CI) of 95% values were also calculated. A p value ≤ 0.05 was considered significant.

Table 1 Frequencies of spondyloarthritis according to gender in 73 Brazilian patients.

Spondyloarthritis	Male *n* (%)	Female *n* (%)	OR (95% CI)	*p*
Mean age ± SD (y)	49.4 ± 12.7	46.2 ± 10.7		0.320[a]
Median age (range)	47.5 (20–80)	48.5 (20–62)		
Ankylosing spondylitis (*n* = 47; 64.4%)	34 (64.2)	13 (65.0)	0.936 (0.328–2.829)	1.000[b]
Psoriatic spondyloarthritis (*n* = 9; 12.3%)	7 (13.2)	2 (10.0)	1.370 (0.259–7.229)	1.000[b]
Undifferentiated spondyloarthritis (*n* = 9; 12.3%)	5 (9.4)	4 (20.0)	0.416 (0.099–1.744)	0.246[b]
Enteropathic spondyloarthritis (*n* = 6; 8.2%)	6 (11.3)	0 (0.0)	5.611 (0.301–10.380)	0.179[b]
Reactive spondyloarthritis (*n* = 2; 2.7%)	1 (1.9)	1 (5.0)	0.365 (0.021-6.141)	0.475[b]
Total (*n* = 73)	53 (100)	20 (100)		

OR = odds ratio; SD = standard deviation.
[a] Calculated by Student *t* test.
[b] Calculated by Fisher's exact test.

Results

Of the 73 selected patients, 53 (72.6%) were male and 20 (27.4%) female. The mean age for the whole casuistic was 48.7 ± 12.2 years and did not differ between the genders ($p = 0.320$). The median for the whole casuistic was 48 years, ranging from 20 years to 80 years. The distribution of the five spondyloarthritis between genders did not present statistically significant differences (Table 1).

Table 2 shows the frequencies of gender and the spondyloarthritis according to the presence ($n = 35$, 47.9%) or absence ($n = 38$, 52.1%) of the *HLA-B*27* gene. The *HLA-B*27* gene was more frequent in males (30/53; 56.6%) than in females (5/20; 25.0%). Ankylosing spondylitis was more frequent in males who were *HLA-B*27* positive (26/30; 86.6%) than in females who were *HLA-B*27* positive (3/5; 60.0%; OR: 10.833, 95% CI: 2.382–49.261, $p = 0.001$; data not shown). The values of OR highlight the importance of the presence of this gene as a risk factor for ankylosing spondylitis in males.

Table 3 shows the frequencies of the clinical signs in patients with and without the *HLA-B*27* gene. The mean age at onset of symptoms was 39.1 ± 11.7 years and did not differ between the genders ($p = 0.905$). A strong association between bilateral sacroiliitis and the *HLA-B*27* gene was observed for ankylosing spondylitis in comparison to other spondyloarthritis (OR: 5.294, 95% CI: 1.474–19.018, $p = 0.009$; data not shown in table). The values of OR highlight the importance of the presence and absence of the *HLA-B*27* gene with the clinical signs of radiological sacroiliitis ($p = 0.014$) and intestinal involvement ($p = 0.048$) among patients suffering from different spondyloarthritis, respectively.

Discussion

The aim of this study was to compare the frequencies of the main clinical signals used as criteria for the diagnosis of spondyloarthritis according to the ESSG, in patients with and without *HLA-B*27*. A sample of patients from the northwestern region of São Paulo, Brazil characterized by the influence of a European background were analyzed.[9] The criteria of the ESSG were adopted for the diagnosis of the spondyloarthritis investigated in this study, since they include clinical, laboratory, imaging, and the presence of *HLA-B*27*. These criteria are considered indicators of good sensitivity and specificity in the diagnosis of this group of diseases.[8]

The HLA genotyping method used in this study is universally recognized as a good tool for characterization of

Table 2 Frequencies of gender, and spondyloarthritis and *HLA-B*27* in 73 Brazilian patients.

Spondyloarthritis	*HLA-B*27* (+) *n* (%)	*HLA-B*27* (−) *n* (%)	OR (95% CI)	*p*
Mean age ± SD (y)	48.5 ± 13.5	48.8 ± 10.9		0.909[a]
Sex				
Male	30 (85.7)	23 (60.5)	3.91 (1.240–12.345)	0.019[b,*]
Female	5 (14.3)	15 (39.5)		
Ankylosing spondylitis	29 (82.8)	18 (47.4)	5.37 (1.813–15.905)	0.003[b,*]
Psoriatic spondyloarthritis	2 (5.7)	7 (18.4)	0.26 (0.051–1.393)	0.155[b]
Undifferentiated spondyloarthritis	3 (8.6)	6 (15.8)	0.50 (0.114–2.175)	0.482[b]
Enteropathic spondyloarthritis	0 (0.0)	6 (15.8)	0.07 (0.003–1.301)	0.025[b,*]
Reactive spondyloarthritis	1 (2.9)	1 (2.6)	1.00 (0.065–18.100)	1.000[b]
Total (*n* = 73)	35 (100)	38 (100)		

+ = presence; − = absence.
* $p < 0.05$ indicates significance.
[a] Calculated by Student *t* test.
[b] Calculated by Fisher's exact test.

Table 3 Main clinical signs in 73 Brazilian patients with different spondyloarthritis according to positivity for the *HLA-B*27* gene.

Main clinical signs	*n*	*HLA-B*27* (+) *n* (%)	*HLA-B*27* (−) *n* (%)	OR (95%CI)	*p*
Axial pain	60	31 (51.7)	29 (48.3)	2.405 (0.667—8.671)	0.226
Radiological sacroiliitis	63	34 (53.9)	29 (46.1)	10.552 (1.260—88.256)	0.014*
Synovitis	27	12 (44.4)	15 (55.6)	0.800 (0.308—2.078)	0.808
Family history	4	3 (75.0)	1 (25.0)	3.469 (0.343—35.039)	0.344
Psoriasis	10	2 (20.0)	8 (80.0)	0.227 (0.044—1.156)	0.088
Intestinal involvement	11	2 (18.2)	9 (81.8)	0.195 (0.038—0.978)	0.048*
Pain in the buttocks	10	3 (30.0)	7 (70.0)	0.415 (0.098—1.753)	0.312
Enthesitis	4	3 (75.0)	1 (25.0)	3.469 (0.343—35.039)	0.344
Diarrhea 1 mo before	1	0 (0.0)	1 (100)	0.352 (0.013—8.938)	1.000
Urethritis	1	1 (100)	0 (0.0)	3.438 (0.131—84.990)	0.475

* $p < 0.05$ indicates significance, calculated by Fisher's exact test.
+ = presence; −, absence.

HLA polymorphisms. It has been extensively used for matching transplant recipients and donors and for genomic wide association studies. It allows the identification of the *HLA-B*27* alleles and the determination of homozygous and heterozygous genotypes with a better level of resolution in comparison with serological methods used in the past, which presented some limitations.[10] Recently, we used this method to determine the frequencies of HLA Class I and Class II genes among voluntary bone marrow donors from the northwestern region of São Paulo.[11]

The *HLA-B*27* gene presented a high frequency in our casuistic, but it was prevalent among those suffering from ankylosing spondylitis. Also, it was prevalent in male patients in comparison to female patients. Despite these differences, there were no statistically significant differences between genders with respect to the frequencies of the six spondyloarthritis analyzed in this study. These data agree with those published by the Ibero-American Registry of Spondyloarthritis (RESPONDIA) Study Group for Brazilian patients and suggest that the frequencies of spondyloarthritis diagnosed in the northwest of São Paulo did not differ from those reported for other regions of Brazil.[12] These observations highlight the clinical importance of the *HLA-B*27* gene as an important immunogenetic risk factor for spondyloarthritis.

The presence of spondyloarthritis was approximately three times higher in men than in women and the average age of onset of symptoms did not differ between the genders. The most frequent spondyloarthritis was ankylosing spondylitis, followed by psoriatic spondyloarthritis, and undifferentiated spondyloarthritis. The enteropathic spondyloarthritis and reactive arthritis appeared less frequently. These data are in agreement with those reported by Gallinaro and colleagues[12] for Brazilian patients. In view of these observations, it can be assumed at least in principle that if there are other selective genetic or environmental factors in nature acting in the genesis of this group of diseases in the region where this study was conducted, they do not seem to modify the frequency of spondyloarthritis commonly diagnosed in the population.

Ankylosing spondylitis, as expected, was positively associated with the *HLA-B*27* gene. The molecular bases underlying this association are not fully understood. However, studies with transgenic animal models show that the *HLA-B*27* gene plays an important role in the genesis of ankylosing spondylitis, but due to the complexity of the disease, this gene is the only one among several genes that determines predisposition and phenotypic variations of this disease.[13] It is possible that incorrect folding and dimerization of the α chain of HLA-B27 glycoprotein in the endoplasmic reticulum, allied to the high expression of interleukin-23 (IL-23) the polymorphisms of its receptor (IL-23R) contribute to susceptibility to ankylosing spondylitis.[3,14—16] These observations support the view that ankylosing spondylitis is a complex disease and although its clinical variability can be influenced by other genes, the *HLA-B*27* gene occupies a prominent position as a marker of susceptibility to this form of spondyloarthritis.[17,18]

The enteropathic spondyloarthritis was negatively associated with the *HLA-B*27* gene, including those patients with manifestations of sacroiliitis. These observations disagree with a previous study[18] but are consistent with others that have shown the absence of the *HLA-B*27* gene in the majority of patients with inflammatory bowel disease associated with spondyloarthritis.[19—22] The reasons for this negative association are not fully understood, but it is possible that ethnicity contributes to the different clinical manifestations of spondyloarthritis in this Brazilian casuistic.[23]

In fact, the population of the northwestern region of São Paulo has a strong Italian, Spanish, Portuguese, and Arabic genetic background, while the sample of Protzer and colleagues[18] is primarily of German origin. Furthermore, it is possible that the absence of the *HLA-B*27* gene exerts a less modulator effect on the genesis of sacroiliitis in the enteropathic spondyloarthritis and that other genes which contribute to modulation of the autoimmune response in this form of spondyloarthritis.[24]

This study noted that radiological sacroiliitis is associated with the *HLA-B*27* gene independent of the type of the spondyloarthritis, but it was stronger for patients with ankylosing spondylitis. This association is explained at least in part by the strong influence of this gene in the pathogenesis of ankylosing spondylitis. The observation that the

severity and the number of sacroiliac lesions correlate strongly with the *HLA-B*27* gene substantiate the proposition that ankylosing spondylitis begins in the sacroiliac joints and progresses to the spine.[17] However, it is not clear how the *HLA-B*27* gene contributes to bilateral sacroiliitis in other spondyloarthritis.[3,17] In fact, a minor proportion of patients with other spondyloarthritis carrying bilateral sacroiliitis and the *HLA-B*27* gene were observed in this study. As the direct effect of this gene on the origin and evolution of sacroiliitis still remains unclear, this topic deserves much more investigation.[25]

This study also observed a marginal association between intestinal involvement and the absence of the *HLA-B*27* gene and these data confirm observations previously reported.[22,26] It is possible that this negative association obscures the radiological manifestations in patients, who displaying other genetic predisposition factors, have sacroiliitis and intestinal injury even in the absence of *HLA-B*27*. Half of the patients analyzed here, carrying the enteropathic form of the disease, presented clinical and radiological signs of sacroiliitis, but they were *HLA-B*27* negative. This observation agrees with the proposition that the presence of sacroiliitis in enteropathic spondyloarthritis is an isolated phenomenon and not related to the *HLA-B*27* gene.[22,26]

All of the six patients with enteropathic spondyloarthritis analyzed in this study first developed inflammatory bowel disease and then had joint involvement. Furthermore, of the 11 patients with intestinal involvement, regardless of diagnosis, nine were *HLA-B*27* negative. The reasons for this sequence of events are not fully understood, but it is possible that the *HLA-B*27* gene has a lower effect in modulating the intestinal autoimmune response in comparison with that affecting the joints, mainly the axial spine.[22,26]

The data presented here must be viewed with caution due to the small number of patients enrolled and therefore these results should be taken as preliminary. Similar studies enrolling greater sample sizes and composed of other ethnic and mixed casuistic in Brazil may contribute to confirming our findings.

Conclusion

In conclusion, we observed that the *HLA-B*27* gene was more prevalent in patients with ankylosing spondylitis, but not in those with enteropathic spondyloarthritis. The image signals of sacroiliitis were associated with the presence of this gene, whereas the intestinal involvement was associated with its absence.

Conflict of interest

All the authors declare that they do not have financial disclosure or conflicts of interest.

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