HLA-B27 and HLA-DRB1 Genotyping of Spondyloarthropathies Among Filipino Patients

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ABSTRACT

Objective To investigate the role of human leukocyte antigen (HLA) genes among Filipino patients of varying ages with spondyloarthropathies compared to healthy controls in a tertiary center.

Methods This is a case-control study where the index patient is matched by a related and unrelated control. HLA-B27 and HLA-DRB1 genotyping were performed via polymerase chain reactions using sequence-specific primers.

Results There were 47 indexed patients with a mean age of 39.38 years, including 22 females and 25 males. Of these, 25 had psoriatic arthritis (PsA), 19 ankylosing spondylitis (AS), 2 undifferentiated arthritis (UA), and 1 inflammatory bowel disease (IBD). More females (64%) had PsA while more males (84%) had AS. HLAB-27 was identified in 22 patients. Among these, 17 were AS patients, 3 PsA, 2 UA, and none with IBD. HLA-B27 was significantly associated with axial involvement (OR = 14, 95%CI 3.38, 58.07) and bilateral sacroiliitis (OR = 16.61, 95%CI 3.11, 88.8), but not with peripheral involvement (OR = 0.125, 95%CI 0.32, 0.485) ($p<0.05$). Of the HLA-B27 + AS patients, 16 had axial symptoms, 14 had bilateral, while 3 had unilateral sacroiliitis, and 3 had uveitis. Of the HLA-B27+ PsA patients, 2 had prominent axial involvement, while 3 patients with axial involvement were HLA-B27-. No pattern of DRB1 alleles was found to be significantly associated with any of the spondyloarthropathies.

Conclusion This first genetic study on genetic polymorphism among Filipino patients strengthened the association of HLA-B27 with AS. However, there was no pattern of association with HLA-DRB1 alleles in this cohort of patients.

This is the first study that confirms a significant HLA-B27 susceptibility of Filipino spondyloarthropathy patients. However, exploratory findings did not find a HLA-DRB1 genotype to extend a similar susceptibility pattern.

Keywords HLA-B27, HLA-DRB1, spondyloarthropathy, genotyping, Filipinos.

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INTRODUCTION AND REVIEW OF LITERATURE

Spondyloarthropathy (SpA) is a family of interrelated conditions that share common clinical (sacroiliitis) and genetic characteristics (HLA-B27) that clearly differentiates it from rheumatoid arthritis and other inflammatory arthritis.

The risk for AS is 16 times higher among HLA-B27 positive relatives than HLA-B27 positive individuals from the general population (1). First-, second-, and third-degree relatives of patients with AS have markedly increased risks of developing the disease (relative risks of 94, 25, and 4, respectively) (2). However, HLA-B27 contributes to only 50% of the total genetic risk of individuals. Studies on AS suggest that additional genetic factors, distinct from the HLA region, are involved. HLA-DR4 contributes significantly to the genetic predisposition to SpA among French families (3). There was a significant association of HLA-DR1 and HLA-B15 with SpA in Mexicans independent of B27 (4). The expression of these genes varied with different forms of SpA.

HLA-B27 Subtypes and SpA Association

More than 27 molecular subtypes of HLA-B27 have been described thus far. HLA-B*2705 is the most widespread HLA-B27 subtype and is clearly associated with AS and related SpAs around the world, except among the West African population of Senegal and Gambia. It is virtually the only observed subtype among the native populations of Eastern Siberia and North America and is present in approximately 90% of the B27 positive individuals of northern European extraction. HLA-B*2704 is the predominant subtype among the Chinese and Japanese and is strongly associated with SpA. In Thailand, B*2704 and B*2706 are equally prevalent, whereas in Indonesia B*2706 is the predominant subtype (5,6).

However, associations with AS appear to differ between subtypes and seemingly between populations as well. Haplotype studies that investigated HLA-B27 interaction with other alleles indicate a subtype-specific AS association for the B*2704 and B*2705 alleles, but not for B*2706 and B*2709 (7,8). A study done on Taiwan Chinese, however, suggests a protective role for B*2705 (9).

Genotyping has been helpful in identifying more patients with the disease and also for recognizing the disease very early. Axial SpA has typically gone undetected until much later in the course of the disease. People with the HLA-B27 genotype have been found to be most susceptible in developing axial SpA.

HLA-DRB1

Despite the established role that HLA-B27 plays in the development of seronegative SpAs, family and twin studies suggest that additional genetic factors may also be involved. Studies on the Caucasian populations suggest DRB1*0401 may be associated with AS in Scots, but no association was found between DRB1*01 or *04 with AS or reactive arthritis in Finns. The DRB1*0408-DQB1*0301 haplotype instead, was noted in HLA-B27-positive Finns suffering from reactive arthritis (10). In Tunisians, a higher frequency of DRB1*15 was found in AS patients, and DRB1*04 in reactive arthritis patients, as compared to healthy controls (11). On the other hand, a study by Komata et al. involving HLA-B27 positive Japanese individuals suggest a linkage disequilibrium between HLA-B27*05 and DRB1*0101 indicating HLA-DRB1 alleles encoding a shared epitope associated with rheumatoid arthritis that may act as an additional susceptibility factor for developing SpA in these individuals (12).

OBJECTIVES

The general objective of this study is to investigate the role of HLA genes among Filipino patients of varying ages with SpAs compared to healthy controls seen at the University of Santo Tomas Hospital. The specific objectives are to investigate whether HLA-B27 is associated with SpA and whether other MHC alleles i.e., HLA-DRB1 is associated with risk for SpAs and severity of disease with HLA genotype.

METHODS

Subjects

This is a case-control study whereby the index patient was matched with both a related and an unrelated control. Demographic profile including serum HLA-B27 positivity, axial and/or peripheral joint involvement, extra-articular features, sacroiliitis, and diagnostics (radiograph/MRI findings) were gathered when available in addition to the physical examination.

Table 1. Demographic Characteristics of 47 Spondyloarthropathy Patients.

	AS (n=19)	PsA (n=25)	USpA (n=2)	IBD (n=1)
Gender ratio (M:F)	16:3	9:16	0:2	0:1
Age at onset (years)	28.28	40.24	30.5	31
Spine involvement	16	4	0	0
Peripheral joint involvement	1	16	2	1
Bilateral sacroiliitis	14	1	0	0
Unilateral sacroiliitis	3	1	0	0
Extra-articular features, i.e., uveitis	3	2	0	0
Family history*	7	4	0	0

**FHx:* includes presence of other rheumatic disease, i.e., SLE, RA, CTDs in first-degree relatives

Genetic Analysis

Genomic DNA from both cases and controls (related and unrelated) were extracted from 4 ml whole blood containing EDTA by standard techniques for HLA typing in the Molecular Genetics Laboratory of the Institute of Human Genetics, National Institutes of Health, University of the Philippines. HLA-B27 and HLA-DRB1 genotyping were also performed via polymerase chain reaction using sequence-specific primers. Genotyping for 18 HLA-DRB1 alleles were done via nested PCR amplification. PCR products were separated via electrophoresis in 2% agarose and visualized using GelRed nucleic acid stain and UV transillumination.

Statistical Analysis

The McNemar test was used to test the equality of proportions between the two paired groups. Equality of odds ratios across age groups was tested using the Breslow-Day test. The magnitude of association was expressed as odds ratio (OR) with corresponding confidence intervals. The level of significance was set at $p = 0.05$.

Ethical Review

The study protocol was reviewed and approved by the independent Institutional Review Board of the University of Santo Tomas Hospital in accordance with the Declaration of Helsinki and the International Conference on Harmonization/Good Clinical Practice.

RESULTS

Forty-seven (47) SpA patients who fulfilled the European Spondyloarthropathy Study Group (ESSG) criteria were recruited. There were 22 females (46.8%) and 25 males (53.2%) with the mean age of 39.38 years ± 15.31 (age range 15-70 years). The spectrum of SpAs include 25 (53%) PsA, 19 (19%) AS, 2 (4%) undifferentiated SA, 1 (2%) IBD, and no patient with reactive arthritis. Most of the patients were of Filipino ancestry while 1.6% (9/47) patients were of Chinese descent. The mean age at disease onset for AS was 28.28 ± 11.42, PsA was 40.24 ± 14.60, undifferentiated SpA was 30.50 ± 10.61, and IBD was 31 years. More females (64%) had PA while more males (84%) had AS. All three cases of IBD and UA were females. There were 23% (11/47) of patients with a family history of connective tissue diseases, i.e. SLE, RA (Table 1). There were 22 females (46.8%) and 25 males (53.2%) with related controls and the mean age of 35.06 ± 18.89, and 25 females (53.2%) and 22 males (46.8%) with unrelated controls and the mean age of 39.38 ±15.31.

Table 2. Association of HLA-B27 Status and Various Spondyloarthropathies

Diagnosed Condition	HLA-B27 Status		Total
	-	+	
AS	2	17	19
IBD	1	0	1
PsA	22	3	25
Undifferentiated SpA	0	2	2
Total	25	22	47

Chi-square = 29.210, df = 3, Asymptotic p-value = .000, Exact p-value = .000

Table 3. HLA-B27 Status of Patients and Related Controls.

		Patients		Total
		HLA-B27+	HLA-B27-	
Related Controls	HLA-B27+	13	0	13
	HLA-B27-	9	25	34
	Total	22	25	47

McNemar Test: p = 0.002
Chi-square = 20.421, df = 1, p = .000
OR cannot be computed since one of the cells has a zero count.

HLA-B27

HLA-B27 was identified in 46.8% (22/47) of patients, 27.6% (13/47) in related controls and 4.2% (2/47) in unrelated controls. Among patients who were HLA-B27+, 77% (17/22) had AS, 14% (3/22) had PsA, and 9% (2/22) UA. HLA-B27 was not seen in the patient with IBD. Eighty-eight percent (22/25) of PsA patients were HLA-B27 negative. (Table 2). Twenty percent (13/47) of patients and their related controls were B27 positive, 19% (9/47) of them were B27+ but B27– related controls, 53% (25/47) of both patients and controls were B27– (Table 3).

Among the clinical presentations of SA, HLA-B27 was significantly associated with axial involvement (OR = 14, 95%CI 3.38, 58.07) and bilateral sacroiliitis (OR = 16.61, 95%CI 3.11, 88.8), but not with peripheral joint involvement (OR = 0.125, 95%CI 0.032, 0.485), with $p<0.05$. However, B27 did not have a significant association with clinical features like shoulder, knee, feet, hand, hip, elbow, and low back pain and uveitis ($p>0.05$). Bilateral sacroiliitis was seen in 15 patients (31.9%), 14 of whom had AS and 1 PsA.

HLA-DRB1 genotype

Of the 94 HLA-DRB1 alleles identified in patients, 25% had DRB1*15, 12% each for *04 and *12, 10% had *0901 while the rest had *08, *1401, *1001, *11, *0701, *16, and *13. No pattern of DRB1 alleles was found to be significantly associated with any of the SpAs.

There were three patients with no identified similar HLA-DRB1 allele with their related controls. Of the 9 patients with exactly the same pair of HLA-DRB1 alleles, 7 were siblings, while 2 were parent and daughter of the index patient. Seventy-four percent of the patients had at least 1 similar HLA-DRB1 allele with their related control.

An exploratory analysis was done on whether HLA-DRB1 gene acts as an additional susceptibility to HLA-B27 positive individuals. However, due to the huge diversity of HLA-DRB1 alleles identified, a statistical analysis could not be deduced from the data.

Ankylosing spondylitis (AS)

Of the 89.5% (17/19) AS patients who are HLA-B27 positive, 94% (16/17) had axial symptoms, 82% (14/17) had bilateral, while 18% (3/17) had unilateral sacroiliitis, and 16% (3/19) had uveitis. Five patients presented with a bamboo spine that contributed significantly to their limited spine mobility. Two patients developed psoriasis, one 5 years after the onset of juvenile AS, while the other patient developed psoriatic skin lesions 2 years after significant spine immobility.

Psoriatic arthritis (PsA)

Mean age of onset of symptoms is much older in patients with PsA compared to AS (40.24 yrs in PsA vs 28.28 yrs in AS). A majority of the patients (20/25) had the peripheral joint more than axial spine (5/25) involvement. There were 4 patients who were HLA-B27+, 2 of whom had prominent axial involvement. However, 3 patients with axial involvement were HLA-B27-. Three patients with significant disability – blindness, hand joint deformity, and limited spine mobility, wheelchair-borne - were all HLA-B27-.

DISCUSSION

To our knowledge, this is the first study to establish the genetic makeup and variation among Filipino patients with spondyloarthropathy. Despite the HLA-B27 genotype commonly implicated in SpA, other MHC classes of genes are equally pointed out in various ethnic groups. A family-based association study done by Eder et al. (13) conferred that HLA-B*27, HLA-B*38, HLA-B*39, and HLA-C*12 alleles are all potential PsA-specific genetic markers among patients with psoriasis. Aside from the HLA-B27 genotype, there is a significant association of HLADR1 and HLAB15 with SpA in Mexicans independent of HLA-B27 (4).

In a random screening done among blood donors in Germany (14), HLA-B27+ donors carry a 20-fold increased risk of developing SpA. AS and USpA are the most frequent SpA subtypes. Patients with inflammatory back pain with HLA-B27+ have a 50% chance of having sacroiliitis. The clinical presentation of HLA-B27 positive patients differs from HLA-B27 negative patients as reported by Chung et al. (15). These patients are associated with a younger age at onset of inflammatory back pain, less delay in diagnosis, lower frequency of psoriasis, and higher frequency of MRI inflammation of the sacroiliac joints. Our study similarly showed a little more than half (9/17) of AS participants who are HLA-B27+ and had their disease onset at <30 years of age. Uveitis as an extra-articular manifestation of AS had been reported to occur between 20-30% in a selected population (16) compared to 16% in our study.

Further to the HLA-B27 gene polymorphism, HLA-B*2701 to HLA-B*2723, Zhen Wu et al. (17) was able to identify B*2704 as the predominant subtype in AS of Han Chinese descent followed by B*2705. HLAB*2715, albeit a rare subtype of the B27 allele, is detected almost only in patients with AS. Of the more than 20 subtypes, *2706 and *2709 are not associated with AS. However, the clinical relevance of the presence of both subtypes in AS is not known.

HLA-B22 was found to be protective in PsA while HLA-B27 in the presence of DR-7, B39, and DQw3 in the absence of DR7 were predictive of subsequent damage (18). In another study, psoriasis and PsA have been associated with HLA antigens B13, B16 (B38, B39), B57, Cw6, and DR7 (19). The frequency of HLA-DRB1*0402 was found to be higher in PsA compared to HLA-DRB1*0401, which was more frequently seen in rheumatoid arthritis patients and controls. Differences in class II HLA epitope in the presence of putative arthritogenic antigen and disease expression remains to be extensively studied (20).

In summary, we did the first study on genetic polymorphism among Filipino patients with SpA. HLA-B27 is found to be strongly associated with AS and similarly reported elsewhere. However, there was no pattern of association of HLA-DRB1 alleles identified in our patients. Further studies are needed to explore the contribution of genetic susceptibility of HLA-B27 genes as well as other genetic markers in detail to clinical disease expression in these patients.

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