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Determination of HLA-B27 Subtypes in Iranian Patients with Ankylosing Spondylitis

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ABSTRACT

The human leukocyte antigen-B27 is one of the class I molecules of the major histocompatibility complex which is strongly associated with ankylosing spondylitis (AS). The strength of the disease association with B27 varies markedly among racial and ethnic populations. It is an allele family, which constitutes about 31 subtypes, with a considerable geographic and ethnic difference in distribution. It is important to know whether certain subtypes show any preferential association with AS. Because there is no report regarding HLA-B27 subtypes in Iranian patients with AS, the main purpose of the present study was to assess the frequency of subtypes of human leukocyte antigen (HLA)-B27 in patients with ankylosing spondylitis in Iranian population

One hundred and nineteen AS patients (82 HLA-B27 positive and 37 HLA-B27 negative) were selected for this study. HLA-B27 positive patients were screened by polymerase chain reaction amplification with sequence-specific primers (PCR-SSP) for B*27 subtyping.

The results of present study revealed that only two subtypes were detected in Iranian patients, including B*2705 (52 patients, 63.4%) and B*2702 (30 patients, 36.6%).

Our results showed a restricted number of HLA-B27 subtypes associated with AS in Iran and an elevated frequency of the B*2705 allele in these patients similar to other Euro-Caucasoid (Aryan) groups in the world.

Key words: Ankylosing spondylitis; Human Leukocyte Antigen; HLA-B27 subtypes; Iran

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INTRODUCTION

The human leukocyte antigen (HLA)-B27 is one of the class I antigens of the major histocompatibility complex strongly associated with ankylosing spondylitis (AS).¹⁻³ AS is considered the prototype of a clinical entities group called seronegative spondyloarthropathies (SNSA), which constitutes a group of disorders with common genetic and clinical characteristics. These disorders are characterized by axial and peripheral arthritis, mainly sacroilitis, and a strong association with the HLA-B27, which is considered an important genetic marker.^{1,4} Although the ethiopathogenesis is unknown, AS seems to be a multifactorial disease, in which environmental and genetic factors could also be involved.⁵

Molecular studies have revealed at least 31 HLA-B27 subtypes with various distributions in different populations.^{1,5} The number of reported HLA-B27 alleles has rapidly increased, most of them differ from each other in a few amino acid residues occupying defined locations in the peptide-binding groove.³

There is a considerable geographic and ethnic difference in distribution of the HLA-B27 alleles.³ B*2701, -2702, -2705, -2708 and -2709 have been reported in Caucasians, whereas B*2704, -2706, and 2707 have been detected only in Asians.^{1,2,6,7} B*2703 is over represented in West African populations and American blacks.^{4,7-10} HLA-B*2705 has been detected in all populations studied around the world and accounts for approximately 90% to 96% of HLA-B27 positive individuals in the Euro-Caucasian (Aryan) groups. This is over represented in circumpolar and sub-artic regions of Eurasia, with a north-south European geographic decreasing gradient. In contrast, there is a southeast-northwest European distribution gradient of B*2702, which is the predominant allele among the Middle East and Jewish populations.^{3,7}

It is important to know whether certain subtypes show any preferential association with AS.³ Mostly, the first ten (B*2701 to B*2710) subtypes were studied for disease association. The predisposition to the disease is reported to be associated with the B*2705, B*2702, B*2704, and B*2707 subtypes.^{1,2,6,7} For the B*2701, B*2703 subtypes, the evidences are controversial, whereas the B*2706 and B*2709 subtypes do not seem to be associated with AS and have been reported to have possible protective role in Thais and Sardinians, respectively.^{6-8,10,11} Other subtypes have not been yet fully studied for disease association. Thus, the definitive conclusion of the disease-associated with particular subtypes requires more ethnic group studies.¹

The Iranian genetic background appears to be a mixture of different populations mainly including the

Euro-Caucasian (Aryan), Arabs and Jews (Semitic) and Mongolians. Aryans, who comprise the main bulk of Iranian population are distributed through out the country. The aim of this study, which was unprecedented in Iran, was to investigate the HLA-B27 subtypes distribution in HLA-B27 positive Iranian AS patients.

MATERIALS AND METHODS

Patients

One hundred and nineteen unrelated AS patients of both sexes (106 males and 13 females), mixed ethnic origins, and residents of Iran, were selected randomly from "Iranian AS Association" during 2005-2006. HLA-B27 ankylosing spondylitis patients were identified by the New York Modified Criteria (MNYC)^{1,4} and they were tested to be the HLA-B27 positive (defined by PCR). HLA-B27 control groups were selected from the data of large population study. All samples were typed at the Department of Immunology, Tehran Medical School. Molecular typing for HLA-B27 alleles (polymerase chain reaction-sequence specific primer [PCR-SSP]) was performed as follows:

HLA Typing

DNA of peripheral blood leukocytes of 119 samples were extracted by modified "salting-out" method.¹² The genetic amplification was performed through PCR. Screening of HLA-B27 accomplished by primers of E2 and E3, which are used to amplify exon 2 and exon 3 of B27 to cover all known B27 subtypes. A primer pair amplifying the third intron of HLA-DRB1 (796bp) was used as an internal control. PCR amplification was done as instructed before.¹³

HLA-B27 Sub-Typing

Typing was performed by sequence specific primer (PCR-SSP) method using "Olerup SSPTM HLA-B*27 Kit" (Olerup SSP AB, Sweden). PCR was performed on a Corbett Thermal cycle, model: CG1-96. PCR products which were visualized in 2% agarose gel under UV illumination following ethidium bromide staining and documented by photography.¹²

RESULTS

From 119 patients with AS, blood samples were provided by Iranian AS Association during a period of one year.

 Table 1. Distribution by age and sex of patients with ankylosing spondylitis

			Male	Female
Age	n=82	%	n=74	n=8
			(90.2%)	(9.8%)
12-22	41	50.00	37	4
23-32	32	39.02	28	4
33-42	7	8.54	7	0
43-52	2	2.44	2	0

 Table 2. Allelic frequencies (f) of HLA-B27 subtypes

 studied in ankylosing spondylitis patients

Allele	n=82	f	Percent
B*2705	52	0.634	63.4
B*2702	30	0.366	36.6

Eighty two patients were HLA- B27 positive as detected with PCR method. The patients' ages ranged from 12 to 52 years. The average age of patients was 23.79 years for males and 22.13 years for females. Among these subjects, 90.24% (74 cases) were males and 9.75% (8 cases) were female (Table 1).

In patients, the frequency of HLA-B27 based on PCR method (Figure 1) was 68.9%. Two HLA-B27 subtypes were detected (Figure 2) in our study, including B*2705, which was the most frequent subtype (63.4%), and B*2702 with a frequency 36.6% (Table 2).

The majority of patients (41 cases) were within the 12-22 years age group, which corresponds with 50% of the total patients. In the same group, 46.15% were B*2705 positive, whereas 56.67% were B*2702 positive.

Table 3. Distribution of HLA-B27 ⁺	ankylosing spondylitis
patients by subtype and age	

Age	Subtype		
	B*2705 (%)	B*2702 (%)	
12-22	24 (46.15)	17 (56.67)	
23-32	23 (44.00)	9 (30.00)	
33-42	4 (7.69)	3 (10.00)	
43-52	1 (1.92)	1 (3.33)	
Total	52 (100.00)	30 (10.00)	

 Table 4. Distribution of HLA-B27⁺ ankylosing spondylitis

 patients by subtype and sex

Subtype	Sex		p-value
	Male	Female	
	n (%)	n (%)	
B*2705	46 (62.2±9)	6 (75±2)	0.4739
B*2702	28 (37.8±9)	2 (25±2)	0.4739
Total	74 (90.2±33)	8 (9.8±33)	

The frequency of B*2705 subtype was $62.2\pm9\%$ in male patients (46 of 74) and $75\pm2\%$ in female patients (6 of 8), thus, the difference was not significant (p<0.4739, Table 4).

The B*2702 subtype was $37.8\pm9\%$ in male patients (28 of 74), and $25\pm2\%$ in female patients (2 of 8). Thus, this difference was not statistically significant as well (p= 0.4739, Table 4).

DISCUSSION

The association between HLA-B27 and AS has been established in 1970s, and remains to be one of the strongest HLA- disease associations which so far has been determined.²



Figure 1. Screening of HLA-B27 subtypes in patients with Ankylosing Spondylitis.

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Figure 2. Determination of HLA-B27 subtypes with PCR-SSP.

The mechanism by which HLA-B27 confers susceptibility to AS is not understood, but is presumed to involve some unique aspect of its role in antigen presentation.^{2,3} The frequency of patients with HLA-B27 varies among different countries, and it is 69.4% in Iran as reported by our previous study¹⁰ and confirmed by present evaluation (68.9%). However, the first report from Iran in this regard had shown a frequency of 92%.¹⁴ The difference between frequencies reported in that study and present study is likely due to the lower number of their patients and to more valid molecular versus serologic technique which we have used.^{12,14}

Genetic heterogeneity of HLA-B27 alleles (subtypes) are increasing due to different population studies and high resolution techniques.⁸ Not all of these alleles are equally associated with AS and are not equally distributed over the world.^{7,9} An example is represented by the Fula ethnic group in Gambia where the frequency of B27 is 6% for both B*2705 and B*2703. However, in this population no case of AS has been reported even though, B*2705 and its relation to spondyloartheropathies (SpA) is widespread in nearly all populations.^{1,7,9,10,15} Moreover, the B*2706 and B*2709 subtypes do not seem to be associated with

AS.^{8,10,11,15,16} On the other hand, B*2701, B*2705, B*2702, B*2704 and B*2707 have been reported to be definitely associated with AS in several studies.^{7,8,10,11,16} B*2708 is a rare European subtype that was first observed in Britain and has been reported in association with AS in a large family from the Azores Islands in the Atlantic Ocean, a territory of Portugal.^{1,6} B*2713 and B*2714 have been found to be associated with AS in Brazilians and Western Indians, respectively.^{6,8} More recently, B*2710, B*2715 and B*2719 have also been reported to be predisposing factors for AS.^{7,8}

Thus, the genetic basis for pathogenesis of the disease in the world population is heterogeneous, with different ethnic groups or populations carrying distinct B27 susceptibility subtypes. These epidemiologic data support the fact that additional genetic factors may influence the disease and that the environmental factors may also contribute to its susceptibility.³

Accordingly, we have found twins with AS one of them was HLA-B27 positive and the other was HLA-B27 negative, while their clinical manifestations of AS were similar. They were 42 years old males living in Tehran. Such findings may, in part, result from differences in co-inherited genetic predisposing factors that may or may not be HLA-linked, or due to the effect of non-genetic (environmental) factors.⁷

According to our results, HLA-B*2705 and B*2702 are the only B*27 alleles detected in the Iranian with B-27-related AS. No significant relationship was found between sex and certain subtypes in this study.

HLA-B*2705 which is the most common allele in the world was found to be the predominant allele (63.4%) among our patients. B*2705 is virtually the only subtype observed among the native population of Eastern Siberia and North America and is distributed in about 90% of the HLA-B27 positive individuals of Northern European descent. This subtype is obviously associated with AS and other SpA.^{1,2,6,7} HLA-B*2702 is the second frequent allele in our patients (36.6%). This allele is present in 4–10% of B27 positive individuals of Northern Europe, 20% in Spain and Portugal and about 55% in Arab and Jewish populations.^{2,6,7,15}

Iranian population is originated from Aryan (Euro-Caucasoid) race who migrated to Persia from Southern Russia about 2500 B.C. Centuries later, through the historical "silk road", many migrations and invasions did take place in this country in several ways, several invaders from and throughout several centuries.¹⁷ According to this fact, one can expect to find more B27 alleles and genetic admixture in Iran, but our results illustrate the homogeneous composition in Iranian patients with AS. The B27 alleles detected in this study are the alleles shared by both Caucasians and North Asian populations (B*2705, 02), and no contribution of others confined to the Middle East and West Asian populations (B*2707), West Africans (B*2703), and North Europeans (B*2708), confirming a strong Euro-Caucasoid (Aryan) component in the population of Iran.

Many factors should be considered before making the definite conclusions, such as sample size, ethnic background, geographic variance, and overall environmental factors. But, the fact that only B*2705 and B*2702 are present in Iranian patients with AS, suggests that this disease, at least in our population, has a Caucasian origin. More extensive typing of Iranian normal population will be necessary to resolve the evolutionary history of HLA diversity in the context of Iranian population demography.

Such subtypes distribution studies in populations with different ethnic origins could provide a theory regarding the involved molecular structures in the disease and clarify the role of HLA-B27 in the AS as well as specific environmental and other possible genetic factors. The subtypes that show the strongest associations within the different ethnic groups and populations may indicate which peptides may contribute the disease susceptibility.

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