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Distribution of HLA-B*27 Alleles in Patients with Ankylosing Spondylitis in Iran

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ABSTRACT

Background: HLA-B*27 is strongly associated with ankylosing spondylitis (AS). It represents a family of alleles that differ among ethnic groups. **Objective:** The aim of this study was to determine the distribution of HLA-B*27 alleles in AS patients and healthy controls in Isfahan (Iran). **Methods:** Sixty AS patients and 430 healthy blood donors were selected. All subjects were HLA-B*27 positive by flow cytometry. HLA-B*27 subtypes were determined by PCR-SSP. **Results:** Forty patients (66.7%) and 17 controls (3.95%) were HLA-B*27 positive. Subtypes detected by PCR-SSP were B*2705, B*2702, B*2704 and B*2707. One patient was B*2702/B*2710. No significant difference was found in the distribution of these alleles between AS patients and controls. **Conclusion:** Although Caucasian subtypes are predominant among Iranians, this population is characterized by a combination of both specific Caucasian and Oriental subtypes. However such results should be interpreted carefully because of the small sample size in our investigation and definitive conclusion awaits more ethnic-group studies.

Keywords: Ankylosing Spondylitis, HLA-B27, PCR

INTRODUCTION

Many investigators have studied HLA-B*27 alleles because of their strong association with ankylosing spondylitis (AS) and related spondyloarthropathies. AS is a chronic inflammatory condition of the spine and sacroiliac joints. It is a progressive disease characterized by a limitation of movement due to an ossification of the interval ligaments. Based on association, linkage, and twin studies, as well as studies of transgenic animal models, strong evidence indicates a direct pathogenetic role of HLA-B*27 in AS.

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In spite of intense research, the exact mechanism of this association remains unclear (1). Strong association of HLA-B*27 with AS was first stated in 1973 (2). Based on epidemiological studies, this association is observed almost worldwide, so that AS prevalence, in general, is proportional to the distribution of HLA-B*27. Its peak is in those populations with highest prevalence of HLA-B*27, and the disease is very rare in sub-Saharan African populations where HLA-B*27 is virtually absent (2).

HLA-B*27 represents a family of alleles with over 31 subtypes (HLA-B*2701–B*2725). In addition B*2722 alleles have been deleted because it was concluded from a sequencing error. These subtypes may have evolved from B*2705 which is the most widespread allele. In spite of some ambiguities, a classification of HLA-B*27 alleles on the basis of their structural features is possible (3).

HLA-B*2705 is the most common B*27 subtype and with the exception of West Africans of Senegal and Gambia, it is associated with AS and related spondyloarthropathies (SPA) around the world (4).

HLA-B*2701 is a very rare subtype observed in Caucasian, Asian, Mestizo, and African American populations. B*2702 is the prevailing allele among Middle East and North African Caucasian populations from Algeria and Tunisia. Its prevalence increases in Southern Europe to 20% in Spain and up to 55% among Arabs and Jews in B*27-positive populations (5, 6). B*2703 has been reported among West Africans (Senegal, Gambia and Mali) and rarely, among African races outside Africa (4, 7). HLA-B*2704 and HLA-B*2706 are the predominant alleles in Asians (8). B*2707 is present at a low level in South East Asians but appears at a higher frequency in West Asia (9). HLA-B*2708 is a rare European subtype that has now been observed in association with AS in a large family from the Azores (10). HLA-B*2709 was originally reported among Sardinians and seems not to be associated with AS. It is interesting that B*2708 and a related subtype, B*2712, which has been observed in two healthy families were serologically quite distinct from other members of the B*27 family (11). HLA-B*2710 is a very rare subtype that was observed only in one Caucasian American family with SPA. B*2711 which has been just reported in one healthy individual from Japan, differs from B*2707 by a single amino acid substitution. B*2713 was observed in a healthy family from Northern Spain and is not indicated in other ethnic groups. B*2714 was described in a Native American individual and was recently identified in association with AS in Western India. B*2719 was observed in a Lebanese Caucasian but no clinical data have been reported on it (5, 12, 10, 13).

The purpose of studying different B*27 subtypes is to learn the structural or functional characteristics common to those subtypes which predispose individuals to AS and SPA. Such knowledge may eventually lead to the discovery of the mechanism by which B*27 contributes to the disease process. One of the strongest reasons for studying the B*27 subtypes is to learn the effects of the sequence variations on the peptide binding specificity of the molecule. Such studies may provide a clue to discovering arthritogenic peptide(s) and may also be useful in studying other pathogenic mechanisms (10).

The question is whether all HLA-B*27 subtypes are among risk factors for AS and if they provide similar or different levels of risk for the disease. In order to answer such questions, distribution of HLA-B*27 subtypes has been determined around the world. These investigations have shown that these subtypes vary in different populations throughout the world because of genetic and geographic differences. Worldwide studies

indicate that HLA-B*2702, B*2704, and B*2705 are strongly associated with AS (13). B*2707 has also been suggested to be associated with the disease except for the Greek Cypriots. However two subtypes, B*2706 and B*2709, seem to lack an association with AS. Other subtypes of HLA-B*27 are too rare to have had disease associations established (8, 13, 14).

These reported ethnic differences among the B*27 subtypes provoked us to examine the distribution of B*27 alleles in Iran. Therefore the aim of this study was to determine the distribution of HLA-B*27 subtypes in the HLA-B*27-positive AS patients and healthy controls from Iran and compare them to other populations.

MATERIALS AND METHODS

Patients and Controls. This work was a case-control study. Based on the modified New York criteria (1), sixty patients with proven AS were referred to the laboratory by a rheumatologist.

In this study, 430 blood donors that had been referred to Isfahan Blood Transfusion Organization were selected as control group. All controls were healthy and without any history of spondyloarthropathies especially AS.

HLA-B*27 Typing by Flow Cytometry. Peripheral blood mononuclear cells (PBMC) were isolated from 2 ml blood sample of AS patients and controls. PBMCs were then stained with FITC conjugated anti-HLA-B*27 and PE conjugated anti-CD3 (Becton Dickinson, California, USA). Lymphocytes were gated according to size and granularity, and analyzed separately. Samples with a median fluorescence 1 (FL1) channel result greater than or equal to the decision marker were considered as HLA-B*27 positive. Samples with a median channel result lower than the decision marker were regarded HLA-B*27 negative. FL1 was a signal from anti- HLA-B*27 FITC and the decision marker was encoded in the suffix of the reagent lot number listed on the vial label of every kit.

HLA-B*27 Subtyping. DNA was extracted from 200 µl blood of HLA-B*27 positive samples by salting out method (15). HLA-B*27 subtyping was done by PCR-SSP kits with 15 reactions (Dynal Biotech, USA). B*2701 to B*2725 (25 alleles) could be detected by this kit. PCR products were electrophoresed on 2% agarose gel and the presence of specific bands was analyzed.

Statistical Analysis. To evaluate the significance of the differences found between patients and healthy controls, Fisher exact test was used. The level of significance was set at 0.05.

RESULTS AND DISCUSSION

This study was performed on 60 patients with proven AS in an age range of 12 to 52 years (average 29.5 years) and 430 age-matched healthy controls. Forty patients (66.7%) and 17 controls (3.95%) were shown to be HLA-B*27 positive using flow cytometry method. These HLA-B*27 positive samples were selected for HLA-B*27 high resolution typing by PCR-SSP. The frequencies of B*27 subtypes in our population with AS and in controls are demonstrated in Table 1. No significant difference was found between AS patients and healthy individuals in the distribution of these alleles. Figure 1 represents a typical gel electropherogram of PCR products.

The association between specific HLA-B*27 subtypes and AS has been detected in different racial groups (2). In this study we assessed HLA-B*27 allele frequencies in Iranian patients (Isfahan) with AS and the results were compared with healthy controls and other populations.

Table 1. Distribution of HLA-B27 subtypes in patients with AS and in controls*

Subtype	Patients(2n=80)[a]	Controls(2n=34)[a]
B*2705	18	5
B*2702	17	7
B*2704	3	2
B*2707	2	3
B*2710	1	0

[a]2n: Number of Chromosomes
*All of the samples were heterozygote for HLA-B locus with one HLA-B*27 allele except one sample in patient group with B*2702/B*2710 genotype.

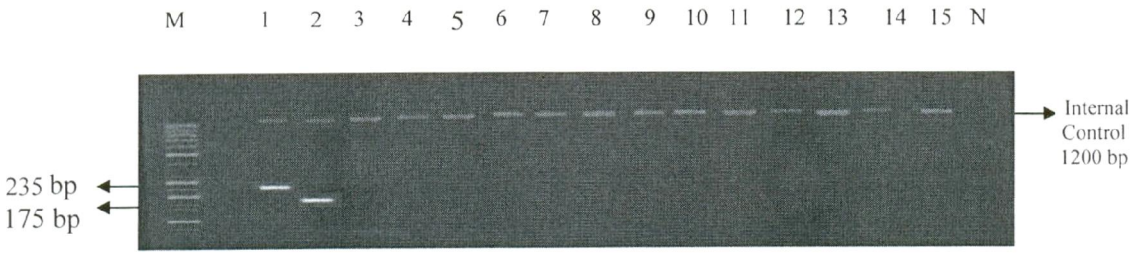

Figure 1. Amplification pattern of B*2705: Lanes 1, 2 show specific bands. (M: Molecular weigh)

Among HLA-B*27 subtypes, B*2702, B*2704, B*2705, B*2707 and B*2710 were identified in our population. According to our results B*2702 and B*2705 were predominant alleles followed by B*2704 and B*2707. All of the samples were heterozygote for HLA-B locus with one HLA-B*27 allele except one sample in the patient group with 2702/2710 genotype. Although Nicknam et al (16), reported that B*2705 and B*2702 were the only B*27 alleles detected in the Iranian AS patients with B*27, we found other subtypes in our AS patients.These differences in the same ethnic group may be mostly due to the highly polymorphic nature of HLA antigens and the small sample sizes

Our finding is in agreement with other studies that demonstrated that B*2705 is the most widespread B*27 subtype around the world (2, 3). Our data also support this finding that there is a clear decreasing gradient in the prevalence of B*2705 from North to the Southeast direction in Asia. (7,8).

Our result is in agreement with previous reports demonstrating B*2702 as a prevalent subtype among Middle East and North African Caucasians. Interestingly B*2702 has been reported to be restricted to the Caucasian populations (6,17).

B*2704 which was another allele found in our population is also a common allele in East Asians and it is the most prevalent subtype in Orientals (18).

In general, HLA-B*27 alleles detected in this study, similar to Garcia-Fernandez study (8), can be classified into two categories: one group of subtypes shared by both Caucasians and North Asian populations (B*2705 and B*2702), another group confined to East (B*2704) and West Asian populations (B*2707). It should be noted that these alleles might be found in other populations.

According to Gonzalez et al (7), each B27 allele is considered to be positively associated to AS if at least one patient in one population has been reported. Instead, some levels of statistical significance must support a negative association.
Most studies, such as those of Asim Khan (6, 13), Gonzalez-Roces (7), Lopez-Larrea (9) and Garcia-Fernandez (8) indicated that B*2705 is clearly associated with AS and related SPA around the world. HLA-B*2702 and HLA-B*2704 are also associated with the disease (7, 9).
HLA-B*2707 has also been proposed to be associated with the disease, except for the Greek Cypriots (19). Our data is supported by other worldwide studies that indicate B*2705, B*2702 and B*2704 to be strongly associated with AS and have similarly confirmed the association of B*2707 with the disease.
Our data support the finding that B*2710 may be a disease associated subtype which needs to be confirmed in additional population studies.
In contrast to the mentioned studies, several investigations have shown that B*2706 and B*2709 are negatively associated with AS (20, 21, 2, 11). As described earlier, these alleles were not found in either patients or controls in our study.
Significant difference was found between AS patients and healthy individuals in the distribution of these alleles in our population as in most other areas. Among various populations, Thais (21), Indonesians (22), Greeks (19), Sardinians (11) and some other rare cases were exceptional and their differences with healthy individuals were significant.
In summary, worldwide studies indicate that occurrence of at least one case of AS or a related SPA has been found in individuals with any of the first 10 subtypes, B*2701 to B*2710 and B*2714 and B*2719, but an association with any of these alleles needs to be confirmed by suitable epidemiologic studies. Lack of evident disease association with some of the other more rare subtypes, may be mostly due to the rarity of these alleles, rather than to their possible lack of disease association (14).
In conclusion, these results indicate that although Caucasian subtypes are predominant in Iranians, this population is characterized by a combination of both specific Caucasian and Oriental B*27 subtypes. However the results should be interpreted with care because of the small sample size in our study and definitive conclusion requires more ethnic-group studies.

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