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Prevalence of HLA-B27 and subtypes of HLA-B27 associated with ankylosing spondylitis in Galicia, Spain

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ABSTRACT

Objective. T*o assess the prevalence of HLA-B27 and its subtypes in both the normal population and in patients with Ankylosing Spondylitis (AS) in Galicia, Northwest Spain.*

Methods. *The prevalence of HLA-B27 in the normal population was determined by checking the number of HLA-B27 positive samples in 308 subjects from different areas of Galicia who had donated organs over a period of 4 years. A total of 106 patients with the diagnosis of AS, according to the modified New York clinical criteria for definitive ankylosing spondylitis, were collected from three very representative areas of Galicia. HLA-B27 was determined by PCR using the primers E91s and E136as, while 11 subtypes of HLA-B27 were analyzed using a commercial kit.*

Results. *The prevalence of HLA-B27 in organ donors was 9.34%. HLA-B27 was present in 94.3% of patients with AS. Subtypes B*2701, B*2709 and B*2710 were not found. The subtypes found in the normal population were; B*2705 (79.5%), B*2702 (18%) and B*2708 (2.5%). The subtypes associated with AS were B*2705 (88%) and B*2702 (12%).*

Conclusion. *The prevalence of HLA-B27 in Galicia was 9.34%, which is higher than previously published in Spain. The frequency of the subtypes associated with AS was similar to that reported for other Spanish regions.*

Introduction

Ankylosing Spondylitis (AS) is an inflammatory rheumatic disease that belongs to the group of the seronegative spondyloarthropathies (1). All the spondyloarthropathies are associated with HLA-B27, although AS is perhaps the disease most tightly linked to HLA. Despite numerous studies conducted in humans and animals, no formal proof exists that HLA-B27 alone is the only gene involved in the pathogenesis of AS (2). Some studies have shown that some genes within the MHC such as HLA-B60 and HLA-DR4 can contribute to disease predisposition. Recent studies have suggested that genes outside the MHC such as those coding for IL-1α, IL-β and IL-1 receptor antagonist may have a linkage with AS (3). Within the next few years, as a result of genome-wide screens, perhaps it will be possible to identify the gene/s involved in the pathogenesis of AS.

Presently there are 25 known subtypes of HLA-B27; they differ among each other by one or a few aminoacid changes, mainly located in the peptide binding site of the HLA-B27 molecule (exon 2 and 3) (4). The exception is the subtype B*2713 that differs from B*2705 in a single base change in exon 1.

HLA-B27 is present worldwide, but the prevalence and distribution of its subtypes differs depending on the ethnicity of the population studied (4). An important step forward in our understanding of these conditions may be to discover whether or not all the known HLA-B27 subtypes are associated with the disease. If a subtype is not associated with the disease, this could allow us to determine the profile of peptides implicated in disease development (5).

In this study we have assessed the prevalence of HLA-B27 and its subtypes in both a healthy population and in patients with AS from Galicia, Northwest Spain. Galicia has a population of 2,700,000 inhabitants. The peculiarities of this population have been reported elsewhere (6); the Galician people have their own regional language and are considered to be of Celtic descent. Previous studies on rheumatoid arthritis have confirmed important immunogenetic differences between the Galician population and other populations from the rest of Spain (6). Therefore, some immunogenetic peculiarities in terms of HLA-B27 association in healthy controls and patients with AS may be anticipated in Northwest Spain.

Patients and methods

Patients

The prevalence of HLA-B27 among the population of Galicia was determined by checking the number of cell cytotoxicity (Terasaki) HLA-B27 positive samples collected from 308 organ donors from different areas of Galicia assessed at Hospital Juan Canalejo (La Coruña) during the years 1996 through 1999 (the Juan Canalejo Hospital is a reference center for transplantation in

the region of Galicia).

As controls, to determine the subtypes in the healthy population, we used HLA-B27 positive samples typed by cell cytotoxicity from normal blood donors; a total of 39 samples could be collected.

A retrospective study reviewing the charts of patients who fulfilled the modified New York clinical criteria for definitive Ankylosing Spondylitis (7) in three highly representative areas of Galicia (La Coruña, Lugo and Orense) was conducted. Clinical data from the medical records was collected according to an established protocol. Patients who did not fulfill the modified New York clinical criteria were excluded from this study. Also, patients who met these criteria but had a clinical diagnosis other than AS such as reactive arthritis, psoriatic arthritis, juvenile spondyloathropathy, arthritis associated with inflammatory bowel disease or undifferentiated spondyloarthropathy were also excluded from this analysis.

A total of 106 patients who fulfilled the modified New York clinical criteria for AS were collected. All ethnically matched controls and patients, regardless of the previous serology data, were typed by PCR for HLA-B27, and later 11 subtypes of HLA-B27 were determined. We verified that healthy controls and patients had a Galician background and could trace their ancestry in the region for at least three generations.

Typing and subtyping of HLA-B27

Blood from AS patients was drawn in the out-patient facilities of the hospitals participating in the study and was frozen in EDTA at –20º. The samples were then delivered by refrigerated transport to the Immunology Department of Juan Canalejo Hospital and stored until the study was conducted. DNA was isolated using the method of denaturation/precipitation with trimethylammoniun bromide salts (Gustincich). To perform the typing of HLA-B27 we used, with some modifications, the single step B27 specific exon 3 PCR assay developed by Domínguez *et al.* (8), and validated by Steffens-Nakken et al (9). We use the primers E91s (5´ GGGTCTCACACCCTCCAGAAT-3´) and E136as (5´-CGGCGGTCCAGG-AGCT-3´) that amplify codons 91-136, which give a final product of 135 bp. The amplification mixture consisted of 100 mM Tris-HCL (pH 9.2), 15 mM $MgCl_2$, 250 mM KCl, 200 ng of genomic DNA, 0.7 mM of each primer (E91s and E136as), 200 mmol of each dNTP and 2 U of Taq Polimerase (Boehringer Mannhein) in a final volume of 50 ml. PCR amplification was performed in a thermal cycler (GeneAmp, PCR System 9600, Perkin Elmer, USA). An initial denaturation was performed at 95ºC for 2 min, followed by 30 cycles: denaturation at 95ºC for one minute, annealing at 65ºC for one minute, and extension at 72ºC for one minute; the final extension was at 72ºC for 5 min. Two previously characterized HLA-B27 positive and negative samples were included in each amplification.

As an internal control procedure for exon 3 amplification, we use GAPDH primers (5´-TGGTATCGTGGAAG-GACTCATGAC-3´) and (5´-ATGCC-AGTGAGCTTCCCG TTAGC -3´) which gives a final product of 190 bp. The PCR products were analyzed by gel electrophoresis on a 2% agarose gel stained with ethidium bromide.

The subtyping of HLA-B27 alleles was performed on all samples, independently of the results of exon 3 amplification; this was done with a commercial kit DYNAL® HLA-B27 "high resolution"-SSP (Oslo, Norway). Presently this allows the identification of HLA-B*2701 through B*27011 alleles. The mixture for the PCR reaction was prepared according to the manufacture's instructions to a final volume of 10 ml. The samples were run in a thermal cycler (GeneAmp, PCR System 9600, Perkin Elmer, USA). The PCR protocol was slightly modified. An initial denaturation was performed at 94º for 45 seconds, 10 cycles were performed at 94ºC for 45 seconds (denaturation) and 66ºC for 75 seconds (annealing and extension). Finally 20 cycles at 94º for 45 seconds (denaturation), 61ºC for 60 seconds (annealing) and 72ºC for 45 seconds (extension) were performed. The PCR products were analyzed by gel electrophoresis on a 2% agarose gel stained with ethidium bromide.

Statistical analysis

The chi-square test, or the 2-tailed Fisher exact test if the expected value was less than 5, were applied for the categorical variables. Statistical significance was defined as $p \leq 0.05$. Calculations were performed uwing the statistical package Stata 8/SE (Stata Corporation, College Station, Texas, USA).

Results

Healthy population

During the years 1996 through 1999 a total of 308 samples were checked (Table I). From these, 6 samples were positive for HLA-B27 in 1996, 9 samples in 1997, 6 samples in 1998 and 8 samples in 1999. Taking into account all the years this yields a prevalence for HLA-B27 in the healthy Galician population of 9.34%. The subtyping of the 39 healthy controls was as follows: 31 were HLA-B*2705 (79.5%), 7 were B*2702 (18%) and one B*2708 (2.5%) (Table I and Fig. 1).

AS population

The distribution of the 106 patients with AS was as follows: 30 patients were from La Coruña, 36 from Lugo and 40 from Orense. The results of the

Table I. Prevalence of HLA-B27 in organ donors in the Galician population.

Year	Nº of samples	HLA-B27+
1996	69	6 (8.69%)
1997	85	9 (10.58%)
1998	74	6 (8.10%)
1999	80	8 (10%)
Total	308	29 (9.34%)

Table II. Subtypes of HLA-B27 in Galicia*.

	Controls (n = 39)	Patients (n = 100)
HLA-B*2705	31 (79.5%)	88 (88%)
HLA-B*2702	7 (18%)	12 (12%)
HLA-B*2708	1 (2.5%)	0

* No statistically significant differences between AS patients and ethnically matched controls were found.

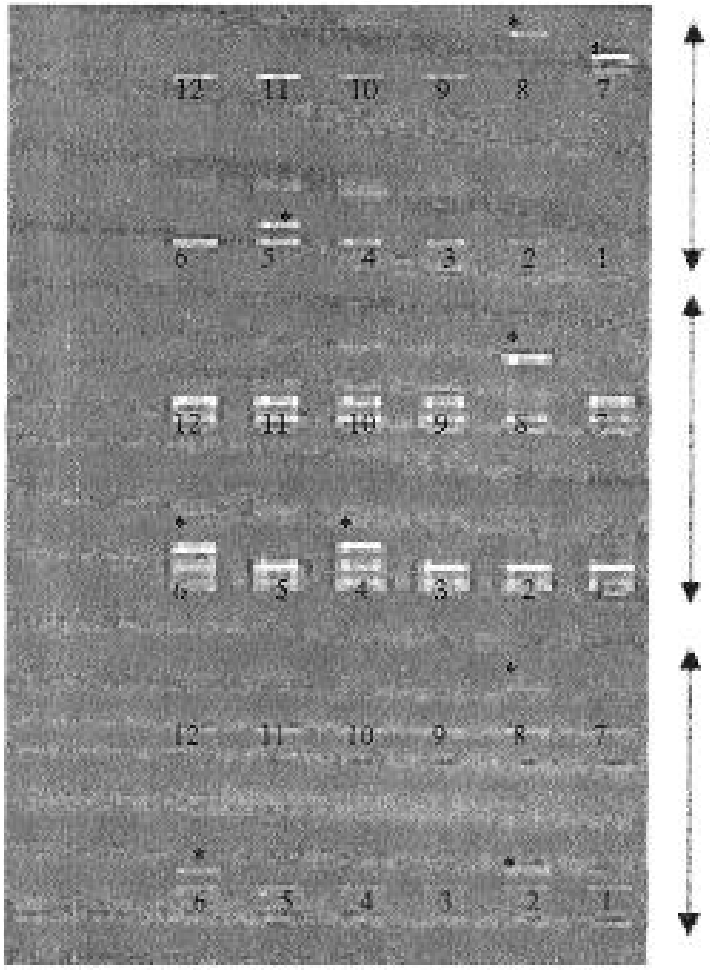

Fig. 1. Each sample of HLA-B27 was run in two lines of 12 lanes. From bottom to top are depicted HLA-B*2702, which was positive in lanes 2, 6 and 8. HLA-B*2705 was positive in lanes 4, 6 and 8, while HLA-B*2708 was positive in lane 5, 7 and 8.

typing of these AS patients by PCR was as follows: 100 patients (94.3%) were HLA-B27 positive and 6 patients (5.7%) were negative. Although primers E91s and E136as cannot identify B*2707 or other subtypes of B27 such as B*2709, 10 and 11, this subtypes are not common in the Caucasian population. Also, to verify and validate the results of the exon 3 amplification we subtyped all the samples. Among the 100 HLA-B27 positive AS patients we found that 88 were HLA-B*2705 (88%) and 12 patients were HLA-B*2702 (12%). No statistically significant differences in terms of HLA-B27 allele association between AS patients and healthy controls were found (Table II). Also, we did not find any false positive or false negative results in the amplification of HLA-B27 with the primers E91s and E136as.

Discussion

The prevalence of HLA-B27 in Europe varies according to the population studied (10). We found a relatively higher prevalence of HLA-B27 (9.34%) in the healthy Galician population compared with other regions of Spain. In this regard, in a study performed in Madrid the prevalence of HLA-B27 was 4% (11), whereas the prevalence in Andalucia, a region in southern Spain, was 4.69% (12). We believe that the heterogeneous genetic background of the Spanish population may account for these discrepancies.

As has previously been shown, PCR amplification of HLA-B27 using the primers E91s and E136as, is a suitable method to identify HLA-B27 (9), and in our study we did not find any false negative or false positive results. The drawback of this method may be the lack of identification of several subtypes that, although rare, are present in the Caucasian population (such as B*2709, B*2710) (10).

The prevalence of HLA-B27 in patients with AS in Galicia was 94.3%. This frequency is in keeping with the current figures of association with AS.

Gonzalez *et al.* have recently described the HLA-B27 associations of AS and related spondyloarthropathies in Asturias, another region in northern Spain (13). These authors identified six different HLA-B27 alleles; B*2705, 02, 03,07,08 and B*2713. In keeping with our observations, in their series on 89 AS patients HLA-B*2705 and 02 were the most common alleles observed in Asturian AS patients. Both HLA-B27 alleles have been found to be most common alleles in former studies on AS in Spain (12, 14).

Gonzalez *et al*. reported an association of the spondyloarthropathies with HLA-B*2708 (13). This allele is a rare subtype identified in the last decade. It has been described in 2 healthy individuals from the British population (15). Also, it was found to be associated with AS in a large family from the Azores Islands (16). In the Galician population B*2708 seems to be very uncommon since it was only identified in 1 of the 39 ethnically matched controls and none of the 100 AS patients exhibited this allele (Table II).

In conclusion, the prevalence of HLA-B27 in Galicia seems to be higher than in other Spanish regions. The frequency of the subtypes associated with AS in Galicia is similar to that reported in other parts of Spain.

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