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Comparative study of the residues 63 and 67 on the HLA-B molecule in patients with Takayasu's Arteritis

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Abstract

Takayasu's Arteritis (TA) has been associated with the Major Histocompatibility Complex (MHC) genes; nevertheless, results in several populations have been heterogeneous. Studies both in Mexican and Asian populations suggest that residues at positions 63 (glutamic acid) and 67 (serine) of the HLA-B molecule could be the genetic markers for TA. In the present work, we analyzed the sequence of HLA-B alleles in 26 TA patients and 62 healthy controls. HLA-B subtyping analysis showed that all B52 alleles were B*5201, whereas only one HLA-B39 allele was B*3902. Sequencing of HLA-B alleles showed that 19 out of 26 patients studied (73.0%) presented at least an allele with glutamic acid at position 63 and serine at position 67. This condition was observed in only 21.0% of the healthy controls (pC = 0.00001, OR = 10.23). Out of the seven remaining patients, one presented glutamic acid at position 63 and four showed serine at position 67. Two patients (2/26 = 7.7%) and 24 healthy controls (24/62 = 38.7%) did not show similarity at the mentioned positions (pC = 0.016, OR = 0.13). These data corroborate the participation of positions 63 and 67 in the genetic susceptibility to TA and explain the high heterogeneity of alleles associated with the disease in several populations.

Keywords: Alleles; Genetic susceptibility; HLA-B molecule; Major Histocompatibility Complex; Takayasu's Arteritis

1. Introduction

Takayasu's Arteritis (TA) is a chronic large vessel vasculitis, which involves elastic arteries such as the aorta and its main branches, and the pulmonary artery in less extent [1]. This disease is more common in Asian and South American individuals that in other ethnic groups, affecting females more frequently than males. Although TA is a disease with unknown etiology, immunological pathogenesis has been suspected for many years. The familial aggregation and association in some cases with immune mediated diseases [2,3] suggest the participation of genetic markers in the development of the disease [4,5]. Some Major Histocompatibility Complex (MHC) genes have been associated to the genetic susceptibility to develop Takayasu's Arteritis in several populations including the Mexican. HLA-B52 is associated with the disease in India, Thailand, Korea, Japan and Mexico; HLA-B39 allele and HLA-B52, DRB1*1501, DRB5*0102, DQA1*0103, DQB1*0601, DPA1*02, DPB1*0401 haplotype have been associated in the Japanese population [6–11]. More recent data note direct participation of the HLA-B

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alleles in the susceptibility to the disease. Data in Asian populations suggest an association with the HLA-B*5201 and HLA-B*3902 alleles, which shared residues at positions 63 (presence of glutamic acid) and 67 (presence of serine) of the molecule [12,13]. Sequencing of HLA-B alleles in Mexican patients show that these alleles share residues with Asian alleles (B51, B52 and B39) associated with the disease [14]. These two positions are important in the training of one of the pockets involved in the antigen-binding site, suggesting that such positions could determine the susceptibility to develop TA by joining and presenting an unknown antigen which could be directly related with the development of the disease. Considering the homology on the residues 63 and 67 of the HLA-B alleles in Mexican and Asian patients, the objective of the present study was to establish the participation of these residues in the genetic susceptibility to TA.

2. Material and methods

2.1. Studied population

The present study included 26 TA Mexican Mestizo patients who attend the Cardiology Outpatient Clinic at the Instituto Nacional de Cardiología Ignacio Chávez, in Mexico City. The diagnosis of Takayasu's Arteritis was based on clinical assessment, laboratory tests and panaortogram angiography. Additionally, a group of 62 non-related healthy individuals with neither symptoms nor previous diagnosis of TA was studied as control group. All study participants were Mexican Mestizos and each individual was asked about their birthplace as well as that of their parents, maternal and paternal grandparents. We considered as Mexican Mestizos only those individuals who, for two generations, including their own had been born in Mexico. A Mexican Mestizo is defined as someone born in Mexico who is a descendant of the original autochthonous inhabitants of the region and of individuals, mainly Spaniards of Caucasian and/or Black origin, who came to America during the 16th century.

The Institutional Ethics and Research Committees approved this study and all subjects signed an informed consent.

2.2. DNA extraction

Genomic DNA from whole blood containing EDTA was extracted by standard techniques [15].

2.3. HLA-B allele typing

Generic variants of HLA-B in the patients were determined by PCR-SSP (polymerase chain reaction with sequence specific primers) (Pel-Freez, Brown Deer, WI, USA) and electrophoresis in 2% agarose gel with ethidium bromide.

2.4. Sequencing of HLA-B alleles

In order to obtain the sequences that span from exon 1 to 3, different 5′ HLA-B specific primers (5′ BE1d, 5′ BE1c) and a 3′ HLA-B and -C specific primer (3′ BCI3) were used to amplify genomic DNA by polymerase chain reaction (PCR). The 5′ primers were designed taking into account the limited polymorphism at exon 1 [16] and each of them is able to amplify specific groups of alleles (Table 1). PCR products from different cells were sequenced by cloning and/or direct sequencing in a Perkin Elmer 310 automated DNA sequencer (Applied Biosystems, AC, USA). PCR sequencing reactions were carried out with the anti-sense 3′ BCI3 [17], the anti-sense 2N [18], and the CG4 [19] primers (Table 1).

2.5. Analysis

HLA-B sequences obtained in the patients were mutually compared with the previously reported for the alleles of susceptibility in the Asian population (HLA-B*5201 and HLA-B*3902 alleles). The frequency of residues in patients and healthy controls was determined using Mantel–Haenzel Chi-square analysis, which was combined with the 2×2 contingency tables using the EPIINFO statistical program (Version 5.0; USD Incorporated 1990, Stone Mountain, GA, USA). Fisher's exact test was used if the number of any cell was less than 5. *P*-values were corrected according to the number of specificities tested and the number of comparisons performed, with a level of significance established as $pC < 0.05$. Relative risk with 95% confidence intervals (CI) was calculated as the odds ratio (OR), according to Woolf's method [20].

Table 1
PCR and sequencing primer sequences

Name	Placing	Sequence 5′–3′	Amplification groups
5′ BE1c	27–44; Exon 1	CCTCCTGCTGCTCTCGGC	B*07, B*08, B*14, B*38, B*39, B*4001, B*4007, B*41, B*4201, B*4501, B*48, B*4901, B*5001
5′ BE1d	−7 to 11; 5′ UT-Exon 1	CGCCGAGATGCGGGTCAC	B*13, B*15, B*18, B*27, B*35, B*40, B*41, B*44, B*4501, 4601, B*4701, B*4901, B*5001, B*51, B*52, B*5301, B*5401, B*55, B*56, B*57, B*58,B*5901, B*78
3′ BCI3	79–96; intron 3	AGCGCTGATCCCATTTTC	PCR and sequencing primer
CG4	157–175; Exon 2	GACGACACCCAGTTCGTGA	Sequencing primer
2N	235–355; Exon 2	GTCCCAATACTCCGGCCCCTC	Sequencing primer