

HLA antigens and juvenile onset spondyloarthritis: Negative association with non-B27 alleles

B. Silva-Ramírez^{1,2}

G. Vargas-Alarcón³

J. Granados^{2,4}

R. Burgos-Vargas^{2,5}

¹Dept. of Population Genetics, Centro de Investigación Biomédica del Noreste, Instituto Mexicano del Seguro Social (IMSS); ²Universidad Nacional Autónoma de México; ³Cellular Biology Section, Dept. of Physiology, Instituto Nacional de Cardiología Ignacio Chávez; ⁴Dept. of Immunology and Rheumatology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán; and ⁵Servicio de Reumatología, Hospital General de México, Mexico City, Mexico.

Beatriz Silva-Ramírez, MSc; Gilberto Vargas-Alarcón, PhD; Julio Granados, MD; Rubén Burgos-Vargas, MD.

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Please address correspondence to: Rubén Burgos-Vargas, MD, Rheumatology Service, Hospital General de México, Dr Balmis 148, México DF 06726, Mexico. E-mail: burgosv@attglobal.net or burgosv@prodigy.net.mx

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ABSTRACT

Objective. To describe the association between HLA-B and HLA-DR genes and juvenile onset spondyloarthritis (SpA) in Mexicans.

Methods. The study included 66 consecutive patients with SpA (45 with ankylosing spondylitis (AS) and 21 with undifferentiated SpA) and 99 non-related healthy controls. The HLA-A, -B and DR alleles were detected by the polymerase chain reaction with the sequence-specific primers technique. Statistical methods included the Mantel-Haenszel χ^2 test, Fisher's exact test, and Woolf method for odds ratio (OR).

Results. The frequency of HLA-B27 was significantly increased in the whole group ($p < 10^{-3}$, OR = 53.0, aetiological fraction = 51%), particularly in AS ($p < 10^{-3}$, OR = 67.42, aetiological fraction 57%). In contrast, the frequencies of HLA-B44, and HLA-B14 were significantly decreased. Also, a weak negative association HLA-DR5 ($p < 0.05$) was found.

Conclusion. Apart from an expected significant association between HLA-B27 and juvenile-onset SpA, particularly AS, we found negative associations with HLA-B44, B14, and DR5. There was also a trend for HLA-B15 and DR1 associations with SpA.

Introduction

The juvenile onset spondyloarthritis (SpA) constitute a group of diseases characterized by peripheral and axial arthritis and enthesitis, which includes juvenile-onset SpA includes ankylosing spondylitis (AS), undifferentiated forms, reactive arthritis and certain forms associated with psoriasis and inflammatory bowel disease (1). Enthesitis related arthritis (ERA) (2) is a term related to the two former clinical forms of juvenile-onset SpA.

Similarly to adult-onset disease, juvenile-onset SpA, and particularly AS are strongly associated with HLA-B27. In addition, some weakly and variable associations with other alleles, including non-HLA-B alleles and non-major histocompatibility complex alleles have been described in several populations (3-6).

In this study, we have further investi-

gated the association between juvenile-onset SpA and HLA Class I and Class II genes in Mexican children.

Patients and methods

The study included 66 consecutive patients with juvenile onset SpA. Twenty-one patients had undifferentiated SpA (7) and 45 AS (8). Fifty-nine patients also fulfilled the ERA subgroup of juvenile idiopathic arthritis diagnostic criteria (2). AS and undifferentiated SpA, not fulfilling ERA criteria, had either a history of psoriasis in first degree relatives or fall into the "undifferentiated arthritis". The control group consisted of 99 non-related healthy subjects with neither symptoms nor previous diagnosis of systemic disease. Both patients and controls, as well as their two previous generations, were born in Mexico. This study was approved by the institutional ethics and research committees and all subjects signed an informed consent.

DNA extraction Genomic DNA was extracted from whole blood containing EDTA by standard techniques (9).

HLA alleles typing Locus A, B, and DR genotyping was performed by polymerase chain reaction with sequence specific primer (Pel-Freez, Brown Deer, Wisconsin, USA). Products were separated by electrophoresis in 2% agarose and visualized by ethidium bromide staining and ultraviolet transillumination. Automated gel reading was performed using Pel-Freez software (Clinical Systems, Brown Deer, Wisconsin, USA).

Statistical analysis Allele frequencies were compared by contingency table analysis using the Mantel-Haenszel ² test excepting those where the number in any cell was < 5 . In such case we used the Fisher's exact test. P values were corrected according to the number of specificities tested and comparisons performed. The level of significance was established at $p < 0.05$. The statistical program "EPIINFO" (version 5.0; USD Incorporated 1990, Stone Mountain, Georgia) was used for these analyses. The magnitude of the association was assessed by odds ratio (OR) and aetiological fractions (AF) statistics (10). Confidence intervals

Table I. HLA allele relevant associations.

Allele	AS (n = 45) n (gf)	Undifferentiated SpA (n = 21) n (gf)	Total (n = 66) n (gf)	Healthy controls (n = 99) n (gf)
B 27	37 (0.578)* OR = 67.42 AF = 57%	14 (0.422)* OR = 35.77 AF = 41%	51 (0.520)* OR = 53.0 AF = 51%	4 (0.020)
B 15	11 (0.130)	3 (0.076)	14 (0.140)	12 (0.060)
B 44	1 (0.011) [†] OR = 0.13	1 (0.023)	2 (0.015) [†] OR = 0.17	16 (0.060)
B 14	1 (0.011)	1 (0.023)	2 (0.015) [†] OR = 0.20	14 (0.070)
DR 1	8 (0.092)	5 (0.127)	13 (0.010)	10 (0.050)
DR 5	5 (0.057)	2 (0.048)	7 (0.05) [†] OR = 0.43	23 (0.120)

*pC < 0.05 versus healthy controls

†p < 0.05 versus healthy controls

OR = odds ratio; AF aetiological fraction (%).

were calculated for ORs by Woolf's method (11).

Results

There were 66 patients (52 boys and 14 girls; mean (SD) age at onset 12.4 (7.2) years) included in the study. Forty-five patients had AS (37 boys and 8 girls; 12.4 (5.4) years) and 21 undifferentiated SpA (15 boys and 6 girls; 13.1 (10.0) years).

Table I shows the distribution of most relevant HLA-B and -DR alleles in patients and healthy controls. The frequency of HLA-B27 was significantly increased in the whole group ($p < 10^{-3}$, OR = 53.0, AF = 51%), particularly in AS (AS, $p < 10^{-3}$, OR = 67.42, AF = 57%). HLA-B15 and HLA-DR1 were also, but non-significantly, increased. In contrast, the frequencies of HLA-B44 and HLA-B14 were significantly decreased when compared with healthy controls (Table I). There was also a weak negative association HLA-DR5 ($p < 0.05$).

Discussion

As expected, we found a significant association between HLA-B27 and juvenile-onset SpA, particularly AS. Interestingly, we also found negative associations between disease and HLA-B44, B14, and DR5, as well as weak associations with HLA-B15 and HLA DR1.

Previous studies of juvenile onset SpA have found associations between HLA-B39 (B*3901) and B60 in the Japanese (3), DR *08, DP 1*0301, and LMP2 in Norwegians (4), as well as LMP2 homozygosity and DR 1*08 in Mexicans (5, 6). The lack of consistency between such studies could result from ethnic factors as well as differences in the study populations, for example the number of patients included in each study. The frequency of HLA-B27 in this study was rather similar to our adult data as well as a trend for increased frequencies of B15 and DR1 (10). In contrast, we have also previously found an association between DR 1*08 in juvenile onset SpA, but not in adult onset disease (6). Despite such differences, juvenile and adult HLA associations in the Mexican population might actually be the same. B2705 is the predominant HLA-B27 subtype in both juvenile and adult onset patients (11).

In Norwegian children with AS, the frequency of the B*4001, DR 1*08, and DP 1*0301 alleles, as well as the LMP2 b/b genotype was significantly increased when compared to B27 positive healthy controls (4). In contrast, the frequency of such alleles in adult onset disease was similar to healthy controls.

The role of non-B27 alleles in increasing the genetic susceptibility to SpA,

but also of those having a protective role has been reported in several populations (12). In this study we found various HLA alleles (HLA-B44, -B14 and -DR5) that might have a protective role in the development of the juvenile-onset SpA. Interestingly, however, is the fact that B*14 was significantly associated with AS susceptibility in West Africans living in Togo (13). Further to ethnic differences, these data might suggest a role for B14 in age at onset. In conclusion, our study confirms an association between HLA-B27 and juvenile SpA. In addition, we found some negative and some possible positive associations with other alleles. Additional studies in a larger number of juvenile-onset SpA patients could help to establish the true significance of these associations in this and other populations.

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