# Association of HLA DRB1 alleles with juvenile idiopathic arthritis in Mexicans

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## Abstract

### Objective

*The aim of the study was to investigate association between HLA class II alleles and juvenile idiopathic arthritis (JIA) in Mexican patients.*

### Patients and methods

*We typed 120 patients with JIA and 99 healthy controls for HLA class II alleles were performed by PCR-SSO. Differences between the whole group of JIA and its subtypes and controls were calculated by using the $\chi^2$; p-values were corrected (pc) with Bonferroni's test.*

### Results

*The alleles HLA-DRB1*01 (pc= 0.00083) and HLA-DRB1*04 (pc=0.0049) were strongly associated with systemic JIA, while HLA-DRB1*11 and HLA-DRB1*14 were found to have decreased frequencies in the patients with systemic JIA compared to the controls. Two alleles were found to have increased frequencies with JIA oligoarthritis subgroup, HLA-DRB1*11 (p=0.01, pc=NS) and HLA-DRB1*13 (p=0.01, pc=NS). The HLA-DRB1*04 was found increased frequencies with susceptibility for RF negative and RF positive polyarthritis JIA subgroups (p correction resulted in loss of significance). In contrast two alleles HLA-DRB1*07 and HLA-DRB1*14 were found decreased frequencies only patients RF positive polyarthritis JIA subgroup compared to the controls (pc=NS).*

### Conclusion

*The profile of HLA-DRB1 alleles associations in Mexican with JIA were somewhat distinct from association typically found in Caucasians.*

**Key words**

Juvenile idiopathic arthritis, HLA-DRB1, molecular class II analysis.

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## Introduction

Juvenile arthritis, whether classified as juvenile rheumatoid arthritis (JRA) (1) or as juvenile idiopathic arthritis (JIA) (2) represents a heterogeneous group of clinical forms conforming the commonest chronic rheumatic conditions in children. According to the ACR criteria, JRA comprises three different types of onset: oligoarticular, polyarticular, and systemic, which correspond to JIA oligoarthritis, polyarthritis (rheumatoid factor [RF] negative and RF positive), and systemic types (2).

Evidence for a genetic basis for JIA has long been described in studies on twins, as well as from concordance for JIA in sibling pairs and mediated by environmental factors (3-5).

Further to variations in the incidence and prevalence of JIA across ethnic and geographically distinct populations (6), various studies have described the association between certain HLA alleles and JIA subgroups. For example, an increase in the DRB1*01 and DRB1*04 alleles has been found in the polyarthritis subgroup in various populations and specifically DRB1*04 with RF positive polyarthritis in older children (7); DRB1*01 with oligoarthritis in younger children extending to polyarthritis (8); finally, JIA has also been associated with DRB1*08 and DRB1*11 alleles (9).

In this study, we aimed to investigate whether HLA-DRB1 and HLA-DQB1 alleles associate with JIA and specific subgroups in Mexican children and, if present, whether such associations resembled those described in other populations.

## Patients and methods

*Patients*

One hundred and twenty Mexican children from two different centers fulfilling the oligoarthritis, polyarthritis (RF positive and RF negative), and systemic JIA International League Associations for Rheumatology classification criteria (2) were included in the study. Except for those with the clinical diagnosis of enthesitis-related arthritis or spondyloarthritis, JIA, HLA-B27 positive children were included in the study. Ninety-nine healthy Mexicans constituted the control group. Both patients and controls were unrelated individuals of Mexican Mestizo ethnic background to the third generation. A Mexican Mestizo is defined as a person who was born in Mexico and whose last two ascending generations were also born in México (10). The percentage contribution from Spanish, Amerindian and African genes in the gene admixture of Mexican Mestizos is 50.3%±4.11%, 49.03%±3.76%, and 0.94%±1.27% respectively (11).

HLA allele typing. Genomic DNA was extracted from whole blood containing EDTA by a modified salting-out technique (12). HLA alleles typing locus DRB1 and DQB1 genotyping was performed by polymerase chain reaction and with sequence-specific-oligonucleotides (PCR-SSO) with kit manufactured by Dynal (Dynal RELI™) and following the manufacturers' instructions.

*Statistical analysis*

Differences between JIA and each of its subgroups and controls were calculated by using the Mantel-Haenzel $\chi^2$ test and Fisher's exact test when appropriate *p*-values were corrected (pc) with Bonferroni's test. The level of significance was established at pc<0.05. The statistical program "EPIINFO" (version 6.0; USD incorporated 1990, Stone Mountain, Georgia) was used for these analyses. Relative risks with 95% confidence interval (95%CI) were estimated as the odds ratio (OR) measured the magnitude of the association (13).

## Results

The JIA group consisted of 120 children classified in the following subgroups: oligoarthritis 36 (30%), polyarthritis 40 (34%), and systemic 44 (36%). Demographic data are shown in Table I.

*DRB1 associations*

Significant increased or decreased gene frequencies of DRB1 alleles are shown in Table II. No significant differences between JIA subgroups and controls in regards to HLA-DQB1 locus were found (data not shown). The alleles HLA-DRB1*11 ($\chi^2$=5.91, *p*=0.01, OR=2.9, CI=1.18-7.04) and HLA-DRB1*13 ($\chi^2$=4.02, *p*=0.04, OR=2.74, CI= 0.99-7.47) were associated with oligoarthritis. Statistical significance,

**Table I.** Patient characteristics and disease subgroups.

| Subtypes | Whole JIA group | Systemic | Oligoarthritis | Polyarthritis, positive RF | Polyarthritis, negative RF |
|---|---|---|---|---|---|
| n (%) | 120 (100%) | 44 (36%) | 36 (30%) | 23 (57.5%) | 17 (42.5%) |
| Male/Female, n (%) | 47/73 (39/61%) | 13/31 (29/71%) | 18/18 (50/50%) | 8/15 (35/65%) | 10/7 (59/41) |
| Age, years, median (range) | 8.7 (1–15.11) | 6.9 (1–15.11) | 9.5 (1–15.11) | 10.5 (3–15.0) | 8.8 (2–13.0) |

**Table II.** Relevant HLA-DRB1 alleles gene frequencies (gf) in Mexican patients with JIA.

| HLA-DRB1 | Systemic (n=88) | | Oligoarthritis (n=72) | | Polyarthritis negative RF (n=34) | | Polyarthritis positive RF (n=46) | | Controls (n=198) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n. | gf (%) | n. | gf (%) | n. | gf (%) | n. | gf (%) | n. | gf (%) |
| 01 | 16 | 18.2<br>$\chi^2$= 11.17<br>pc= 0.00083<br>0R= 4.18<br>CI= 1.68-10.75 | 4 | 5.5 | 2 | 5.9 | 4 | 8.6 | 10 | 5.0 |
| 04 | 36 | 40.9<br>$\chi^2$= 7.91<br>pc=0.0049<br>OR= 2.22<br>CI=1.25-3.93 | 18 | 25 | 15 | 44.1<br>$\chi^2$=5.16<br>*p*=0.02<br>OR=2.54<br>CI=1.10-5.72 | 18 | 39.0<br>$\chi^2$=3.77<br>*p*=0.05<br>OR= 2.07<br>CI=0.98-4.26 | 47 | 23.7 |
| 07 | 4 | 5.4 | 4 | 5.5 | 2 | 5.9 | 1 | 2.1<br>$\chi^2$=2.29<br>F=0.05<br>OR= 0.19<br>CI=0.00-1.23 | 21 | 11.0 |
| 11 | 1 | 1.1<br>$\chi^2$=3.21<br>*p*=0.02<br>OR=0.15<br>CI=0.0-1.03 | 13 | 18.0<br>$\chi^2$=5.91<br>*p*=0.01<br>OR= 2.90<br>CI= 1.18-7.04 | – | – | 2 | 4.3 | 14 | 7.0 |
| 13 | 4 | 4.5 | 10 | 13.9<br>$\chi^2$=4.02<br>*p*=0.04<br>OR= 2.74<br>CI=0.99-7.47 | 2 | 5.9 | 3 | 6.5 | 11 | 5.5 |
| 14 | 3 | 3.4<br>$\chi^2$=4.86<br>*p*=0.027<br>OR=0.24<br>CI=0.05-0.84 | 9 | 12.5 | 1 | 2.9 | 1 | 2.1<br>$\chi^2$=3.38<br>*p*=0.02<br>OR=0.15<br>CI=0.00-0.97 | 25 | 12.6 |

OR: Odds ratio; CI: Confidence interval.

however, is lost after Bonferroni correction of the *p*; a similar trend was found between HLA-DRB1*04 and RF negative ($\chi^2$ =5.16, *p*=0.02, OR=2.54, CI=1.10-5.72) and RF positive polyarthritis ($\chi^2$ =3.77, *p*=0.05, OR=2.07, CI=0.98-4.26). In contrast, two alleles HLA-DRB1*07 and HLA-DRB1*14 ($\chi^2$ =2.29, *p*= 0.05, OR=0.19, CI=0.00–1.23 and $\chi^2$ =3.38, p=0.02, OR=0.15, CI= 0.00–0.84 respectively) appeared protective against RF positive polyarthritis. Systemic JIA was significantly associated with HLA-DRB1*01 ($\chi^2$=11.17, pc=0.00083; OR=4.18, CI= 1.68–10.75) and HLA-DRB1*04 ($\chi^2$=7.91, pc=0.0049, OR=2.22, CI=1.25-3.93) and HLA-DRB1*11 and HLA-DRB1*14 appeared protective against systemic JIA ($\chi^2$=3.21, *p*=0.02, OR=0.15, CI=0.00–1.03 and $\chi^2$ =4.86, *p*=0.027, OR=0.24, CI=0.05–0.84 respectively).

## Discussion

Diverse studies have linked genetic susceptibility to JIA. The stronger associations have been found with HLA-DR and HLA-DQ regions, but there are considerable variations in their with JIA subtypes across ethnic groups. In part, such variations may be related to genetic differences between ethnic groups and perhaps differences in disease classification.

Similarly to Caucasian and Colombian Mestizo studies (9, 14, 15), HLA-DRB1*11 and HLA-DRB1*13 alleles tended to be associated with susceptibility to oligoarthritis in our group. Interestingly, HLA-DRB1*11 has also been associated with persistent oligoarthritis (16) and HLA-DRB1*13 with chronic uveitis (17, 18). Similarly to Pazár *et al.* (19) in the Hungarian population, HLA-

DRB1*11 was also the most frequent allele in 12 patients with persistent oligoarthritis in our study. In contrast, HLA-DRB1*13 was not associated with uveitis.

The well established association between RF positive polyarthritis and HLA-DRB1*04 (20) was also found in Mexicans with RF positive and also negative polyarthritis. Interestingly, this latter association has been also described by Garavito *et al*. in Colombian Mestizos (15). Contrary to Europeans (14, 19, 21, 22), the HLA-DRB1*08 allele was not associated with RF negative polyarthritis in this study. As described in some Caucasian populations, we found HLA-DRB1*07 and HLA-DRB1*14 decreased in both RF positive and negative polyarthritis. These findings probably reflect a protective effect against polyarticular JIA, or protective allele against autoantibody production (19, 23, 24).

One of the most interesting findings was the strong positive association between HLA-DRB1*01 and HLA-DRB1*04 and HLA-DRB1*11 and HLA-DRB1*14 negatively associated with systemic JIA. While HLA-DRB1*04 has been consistently associated with susceptibility to systemic JIA in most studies (25-27), the HLA-DRB1*01 allele has been associated with an increased risk of enthesitis-related arthritis and polyarthritis in Caucasians children (22). Recently, Shishov found an increased number of joints involved in systemic onset JRA in Mexican children at the onset of disease (28). While HLA-DRB1*11 was protective for polyarthritis and systemic JIA, it seemed to be associated with susceptibility to oligoarthritis.

We acknowledge that in this study, most associations were certainly weak, probably as consequence of the small number of patients. It would be also possible that the genetic background of patients from one center differed from the other since the north of country was occupied by Amerindians who were different from those in the central part. Nevertheless, we may conclude that the profile of HLA-DRB1 association with JIA resembles those found in Caucasians. Differences, however, support the notion that JIA susceptibility to certain subtypes across ethnic groups depends on the presence of specific alleles that either predispose or protect the individual

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