

## Role of human leukocyte antigen DRB1\*0307 and DRB1\*0308 in susceptibility to juvenile rheumatoid arthritis

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### ABSTRACT

**Objective.** To study the prevalence of Human Leukocyte Antigen (HLA) DR alleles in children with juvenile rheumatoid arthritis (JRA).

**Methods.** DNA samples from 64 children with oligoarticular and seronegative polyarticular JRA and 64 controls of the same ethnic background were analyzed using PCR-sequence specific primers (PCR-SSP) method. Analysis took into account the onset subtype, the presence of antinuclear antibodies (ANA) and the presence of chronic anterior uveitis, a recognised serious complication of JRA.

**Results.** A high prevalence of DR3 alleles were detected in children with oligoarticular JRA compared to controls ( $p < 0.05$ ). DR3 alleles were the commonest also in patients with positive ANA as well as those with chronic anterior uveitis. The interesting finding in this study is the absence of two DR3 alleles, namely DRB1\*0307 and DRB1\*0308 in the control group while present in significant proportion in children with JRA. DRB1\*0307 was present in 16% of children with oligoarticular subtype and 15% of those with polyarticular JRA. DRB1\*0308 was only detected in children with oligoarticular JRA, none of the children with polyarticular JRA or the controls had this allele.

**Conclusion.** These findings support earlier observations linking these two DR3 alleles, namely 0307 and 0308, to the genetic susceptibility to JRA.

### Introduction

Juvenile rheumatoid arthritis (JRA) is a heterogeneous chronic disease with variability in its presentation, course, extra-articular manifestations and long-term prognosis (1, 2). The disease is classified on the basis of presentation during the first six months from its onset into pauciarticular onset or oligoarticular (with less than 5 joints involved at onset), polyarticular (5 or more joints) and systemic (3-7). Patients with JRA, particularly girls with oligoarticular disease, develop chronic anterior uveitis (4). Apart from uveitis, extra-articular manifestations are unusual in oligoarticular but common

in polyarticular and systemic disease. Generally, patients with the rheumatoid factor (RF) positive form are considered a separate group, with a similar genetic predisposition to rheumatoid arthritis seen in adult patients. Also excluded from JRA are cases with spondyloarthropathy, psoriatic arthritis or other known causes of chronic arthritis.

Several previous studies have reported an association of JRA with different human leukocyte antigen (HLA) class I and class II alleles, which underlines the clinical and immunological heterogeneity of the disease. Using serological typing methods, HLA-DR8 and DR2 were found to confer susceptibility to this form of the disease while DR4 and DR7 antigens were found to be protective (8). Using DNA typing methods the oligoarticular type was shown to be associated with DRB1\*0801 and DRB1\*1301 haplotypes (8, 9). Ploski showed that DR3, 5 or 6 together with DPB1\*0201 conferred susceptibility in Norwegian JRA patients (10). However, no association was detected for DRB1\*08 allele in a study from Germany (11), nor from a large study from Greece (12). Donn & Ollier have reported a high prevalence of DRB1\*0801, \*1101 and \*1301 alleles in early onset oligoarticular JRA (13). Still others have failed to show any significant association with HLA-DR alleles in their population (14). Recently we have reported an interesting association with two of the DR3 alleles namely DRB1\*0307 and DRB1\*0308 in Kuwaiti Arab children with RF negative oligoarticular and polyarticular subtypes of JRA (15). The high prevalence of the specific DR3 alleles (DRB1\*0307 and \*0308) have accounted in part for the genetic susceptibility to these forms of JRA in our patients.

In order to further study the significance of our finding, we analysed the prevalence of HLA-DR alleles including the different DR3 alleles in children with JRA from another racial background. We have included in the present study only Caucasian children with RF negative oligoarticular and polyarticular JRA and racially matched controls.

### Subjects and methods

### Patients

HLA-DRB1 genotyping was performed on 64 white children with JRA who were seen on regular basis in the rheumatology outpatient clinic of the University of Florida with the diagnosis of JRA made at least 6 months before the initiation of the study. All patients are from Caucasian families residing in the region of North Florida. Detailed clinical information was available on all patients, including gender, age of onset, joints affected, presence of enthesitis, presence of extra-articular manifestations including iridocyclitis, and ANA and RF test results. The patients were classified according to the American College of Rheumatology 1987 criteria. Inclusion criteria were the presence of oligo- or polyarthritis. Patients with a positive rheumatoid factor test, enthesitis or history or a family history of psoriasis or inflammatory bowel disease were excluded. Only patients with oligoarticular and rheumatoid factor negative polyarticular JRA course were included. All patients were examined at 4-6 monthly intervals by an ophthalmologist for anterior uveitis. HLA-DRB1 allele frequencies in JRA cases were compared with a group of 64 control children with a similar ethnic background and from the same geographical region. Verbal informed consent was obtained from all patients included in this study. The antinuclear antibody in the serum was detected by immunofluorescence as described by Donn; a titer of 1:40 or more was considered positive (16). Rheumatoid factor in the serum was measured by nephelometry (Beckman Array Nephelometer) according to manufacturer's recommendation.

### Genotyping

Blood samples were collected from patients and control children after obtaining verbal consent. Total genomic DNA was isolated by a standard procedure as described by Sambrook (17). HLA-DR alleles were identified by a commercial kit (Dynal, Oslo, Norway) which utilizes a DNA-based PCR-SSP method described by Olerup (18). The technique is based on the principle that a completely matched primer will be

more efficiently used in PCR reaction than a primer with one or several mismatches in the 3' end. Allele specific PCR products were analyzed by gel electrophoresis followed by staining with ethidium bromide. All analyses were carried out double blind and appropriate positive and negative controls were included for each sample run.

### Statistical analysis

The frequencies of DRB1 alleles were calculated by a standard method. Throughout this report, the term "frequency" refers to the phenotypic frequency. The significance of the differences in allele/phenotypic frequencies was evaluated by chi-squared analysis with Yates correction.

### Results

The patient group consisted of 64 children, of whom 51 had the oligoarticular and 13 the polyarticular variety of JRA; all patients tested negative for rheumatoid factor. The oligoarticular group consisted of 37 girls and 14 boys while the polyarticular group consisted of 10

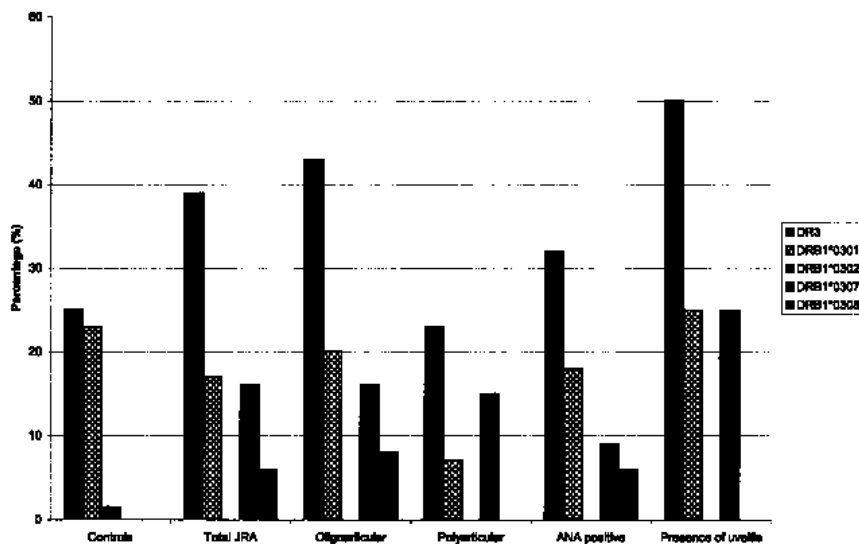
girls and 3 boys. The frequency of the HLA-DR alleles were analyzed for the polyarticular form alone, oligoarticular alone and the two subtypes together. The allelic frequency in each of the 3 groups was compared to the frequency in a control group consisting of 64 healthy children. Among the patients, 34 were positive for antinuclear antibody (53%) and 8 patients were diagnosed with chronic anterior uveitis (12.5%).

The frequency of HLA-DRB1 alleles determined by the DNA-based PCR-SSP method is shown in Table I. Figure 1 demonstrates the percentage of patients with DR3 and its alleles in the controls and the different patient groups, i.e. oligoarticular, polyarticular, all JRA, ANA-positive, and those with uveitis. The incidence of all the tested DR3 alleles were higher in the group of patients with oligoarticular disease compared to the control group (43% versus 25% respectively,  $p < 0.05$ ). Two of the DR3 alleles, namely DRB1\*0307 and DRB1\*0308, were not detected in any of the control sub-

**Table I.** HLA-DR frequencies in JRA patients and normal controls.

DR-type	Controls N = 64 N (%)	Oligoarticular N = 51 N (%)	Polyarticular N = 13 N (%)	Total JRA N = 64 N (%)
DR1	11 (17.2)	12 (23)	3 (23)	15 (23)
DR3	16 (25)	22 (43)**	3 (23)	25 (39)
DRB1*0301	15 (23)	10 (20)	1 (7)	11 (17)
DRB1*0302	1 (1.5)	0	0	0
DRB1*0307	0	8 (16)	2 (15)	10 (16)
DRB1*0308	0	4 (8)	0	4 (6)
DR4	9 (14)	8 (16)	3 (23)	11 (17)
DR7	10 (16)	11 (22)	2 (15)	13 (20)
DRB1*0701	10 (16)	11 (22)	2 (15)	13 (20)
DR8	1 (1.5)	5 (10)	0	5 (8)
DR9	1 (1.5)	2 (4)	1 (7)	3 (5)
DR10	2 (3)	0	1 (7)	1 (1.5)
DR11	20 (31)	4 (9)	3 (23)	7 (12)
DR12	2 (3)	4 (8)	0	4 (6)
DR13	14 (22)	11 (22)	2 (15)	13 (20)
DRB1*1301	14 (22)	11 (22)	2 (15)	13 (20)
DR14	4 (6)	5 (10)	1 (7)	6 (10)
DR15	21 (33)	11 (22)	4 (31)	15 (23)
DRB1*1501	21 (33)	11 (22)	4 (31)	15 (23)
DR16	2 (3)	1 (2)	0	1 (2)
DRB1*1601	2 (3)	1 (2)	0	1 (2)

\*\*  $p < 0.05$  as compared to controls



**Fig. 1.** The prevalence (%) of DR3 and its alleles in the controls and patients (total JRA patients, oligoarticular subtype, polyarticular subtype, ANA positive and the presence of uveitis, respectively).

jects; in contrast DRB1\*0307 and DRB1\*0308 were detected in 10% and 5% of the JRA patients, respectively. 16% of patients with oligoarticular JRA and 15% of children with polyarticular disease had the DRB1\*0307 allele. None of the controls nor the children with polyarticular JRA had the DRB1\*0308 allele, while 8% of those with the oligoarticular subtype tested positive for this allele. DRB1\*08 was higher in patients with oligoarticular JRA but did not reach a significant level, while DRB1\*04 was present in higher frequency in the polyarticular group but without statistical significance.

Of the 8 children with uveitis, 4 had the DR3 alleles (2 were DRB1\*0301 and 2 were DRB1\*0307), 2 were positive for DRB1\*1101, and 2 were positive for DRB1\*1301. When patients were analyzed according to the presence of anti-nuclear antibody (ANA), 34 tested positive (53%). Among all the DR alleles tested in this study, DR3 alleles were the commonest alleles detected in the group of patients with a positive ANA test. Of all patients with a positive ANA, 11 were DR3 positive (32%), of those 6 had DRB1\*0301 (18%), 3 had DRB1\*0307 (9%) and 2 had DRB1\*0308 (6%).

### Discussion

HLA has long been thought to be relat-

ed to the pathogenesis of JRA. Reported associations between JRA and HLA alleles are variable, partly due to the methods used for analysis and partly to the ethnic groups studied. The important finding in this study is the association of DR3 alleles, namely HLA-DRB1\*0307 and DRB1\*0308, with the oligoarticular subtype of JRA. This is not a novel finding, as it has been described in some earlier reports. Ploski reported an association of DR3 with DPB\*0201 in JRA patients from Norway (10). We have also reported in a previous study a similar association with DR3 alleles in Kuwaiti children with JRA (15). In that study two DR3 alleles, DRB1\*0307 and \*0308, were found to account for the increased incidence of DR3 in the JRA patients. While in this study DR3 was found to be associated with oligoarticular JRA, in accordance with previous reports, a significant finding was the incidence of the two DR3 alleles DRB1\*0307 and DRB1\*0308. The DRB1\*0308 allele was only present in the oligoarticular group, and was not detected in the control nor the polyarticular group. The DRB1\*0307 allele was also absent from the control group but accounted for 16% and 15% of the oligoarticular and the polyarticular subtypes respectively. In the control group HLA-DRB1\*0301 was the predominant DR3

allele detected. Statistical analysis comparing the incidence of DRB1\*0307 and DRB1\*0308 in the patients and controls was not technically feasible because of the absence of these alleles in the control group; nevertheless, we believe that this is an important and relevant finding for understanding genetic susceptibility in this subgroup of JRA.

Of the remaining DR alleles analyzed, HLA-DR8 (DRB1\*0801) was more frequent in the patients with oligoarticular JRA than in the control group. This represents a known and consistent association with oligoarticular JRA (8). Interestingly, none of the children with polyarticular JRA carried the DRB1\*0801 allele. On the other hand, DRB1\*0401 was more common in this subtype of JRA. In the control group DR 15 was found to be more prevalent, but no allele was found to be protective against JRA in our present study.

DR3 alleles were also the commonest alleles in patient with ANA positive JRA, perhaps adding to the importance of DR3 in the genetic susceptibility to JRA as a consistent finding. Despite the relatively small number of patients with uveitis, DR3 alleles were also the commonest detected. This is unlike the previously reported association mainly with DRB1\*1104 in children with this serious complication of JRA (19).

The difference in the HLA-DR alleles found to be associated with JRA should not imply a lack of importance when linking inheritance of HLA to susceptibility to JRA. In fact, these findings are not unlike those reported for the adult form of rheumatoid arthritis where more than one allele is associated with a single well-defined entity of the disease. Rheumatoid factor positive adult rheumatoid arthritis has been associated with the DRB1\*0401, \*0404, \*0405, \*0408, \*0101, \*0102 and \*1001 alleles. These alleles were found to encode a specific epitope shared among these alleles. Indeed it was found that the effect of these shared epitopes conferred the risk in a dose-dependent way (20, 21). DR3 alleles have in common with the other alleles linked to oligoarticular JRA that they too are included in the DR52 super-specificity.

Prahalad used a different analytical method to demonstrate the linkage of oligoarticular JRA with HLA-DR and the alleles most likely involved. He studied allelic sharing in 80 pairs of affected siblings, and showed that affected sibpairs with JRA share HLA-DR alleles significantly more often than expected, providing additional evidence for a linkage between the HLA region and JRA. Excess allelic sharing was again demonstrated in affected sibpairs who were concordant in their JRA course, whether this course was oligoarticular or polyarticular. HLA-DR8 was shared significantly more frequently in the affected sibpairs. HLA-DR pairs linked to JRA included DR 8/11 and DR8/3 (22). Both DR8 and DR3 were also increased in our patients with oligoarticular JRA.

Ploski found a statistically significant increase of DPB1\*0201 among early onset oligoarticular JRA patients carrying at least one DRB1 allele encoding DR3, DR5 or DR6, although in their data none of the DRB1 alleles showed a statistically significant interaction with DPB1\*0201 when analyzed separately (10). Van Kerckhove and Ploski suggested that DR3, 5 or 6 and DPB1\*0201 can potentiate each other's effects whereas, in contrast, the effect of DR8 is independent from any interactions (10,23). This, they thought, may suggest that a difference exists in the mechanisms by which DR8 on the one hand, and the DPB1\*0201 allele together with DR3, 5 or 6 on the other, predispose to early onset oligoarticular JRA. It has been postulated that for complex traits like JRA the differences in immune responses directed towards self antigens are likely to be central to the pathogenesis of the disease. This responsiveness will most likely be determined by the particular array of genes present in an individual, i.e. specific HLA alleles may favour the presentation of one peptide antigen over another to a specific T-cell repertoire predetermined genetically at the thymic level (at least in part by HLA alleles as well) and modified by the presence of null allele, in particular the

T-cell receptor (TCR) families. A characteristic of such abnormal immune responses is the development of specific autoreactivity to self antigens such as heat shock protein (HSP) and the nuclear protein DEK antigens, although whether the response to these antigens is disease-promoting or instead protective is unknown in the setting of different forms of JRA (24). However, on the basis of our data from children with JRA, it would be fair to suggest that an individual with DR3 (DRB1\*0307 and \*0308 alleles) would have a somewhat higher degree of predisposition for the disease, although the study must be extended to larger numbers.

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