

## CONCISE REPORT

# Juvenile idiopathic arthritis (JIA) is primarily associated with HLA-DR8 but not DQ4 on the DR8-DQ4 haplotype

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**Background:** Juvenile idiopathic arthritis (JIA) is strongly associated with the DR8-DQ4 haplotype. The genes encoding DR8 and DQ4 are in strong linkage disequilibrium (LD) and occur together on the same HLA haplotype in almost all patients and controls. Because of the strong LD it is not clear whether DR8, DQ4, or both, are primarily associated with JIA.

**Objective:** To unveil the primary association of JIA—that is, with DR8 or DQ4.

**Methods:** DRB1, DQA1, and DQB1 alleles of 585 Norwegian and 47 Polish unrelated patients with JIA (categorised as pauciarticular and rheumatoid factor negative polyarticular JIA), and of 3155 Norwegian and 158 Polish unrelated controls, were typed using a polymerase chain reaction or oligonucleotide hybridisation and sequence-specific primers method.

**Results:** Several haplotypes which encoded DR8 (that is, carried DRB1\*08) and which did not encode DQ4 (that is, did not carry DQA1\*0401) were found. Such haplotypes were found in three Norwegian patients and two controls ( $p=0.029$ ). In the Polish population such haplotypes were found among four patients with JIA and two controls ( $p=0.025$ ). No haplotypes which carried DQA1\*0401 and DQB1\*0402 in the absence of DRB1\*08 were found, either among patients with JIA (Polish and Norwegian) or among the controls (Polish).

**Conclusion:** On the DR8-DQ4 haplotype the DRB1\*08 allele is primarily associated with JIA.

Juvenile idiopathic arthritis (JIA) has been found to be associated with several HLA alleles.<sup>1–3</sup> Among the HLA class II associations, the strongest and the most consistently observed is the association with the DRB1\*08, DQA1\*04, and DQB1\*04 alleles, encoding the DR8 and DQ4 molecules, respectively. These associations have been observed in pauciarticular JIA, rheumatoid factor (RF) negative polyarticular JIA groups,<sup>1–5</sup> and in juvenile ankylosing spondyloarthritis,<sup>6</sup> which suggests that they may represent markers of seronegative arthritis in children.

Among white subjects, the DRB1\*08, DQA1\*04, and DQB1\*04 alleles are in strong linkage disequilibrium (LD) and virtually always occur together, forming the DR8-DQ4 haplotype.<sup>7</sup> As this strong LD is found among both patients and controls, it is difficult to determine which HLA class II genes are primarily involved in JIA; those encoding DR8 or DQ4, or both.

Two approaches have been used to determine whether DRB1\*08 or DQA1\*04 is primarily associated with JIA. The first relies on an analysis of the frequency among patients and controls of “rare” haplotypes, which encode DR8 without DQ4, or vice versa. Although this is a direct way to address the problem, it has not given clear results, probably because relatively small cohorts of patients and controls have been

analysed. Nepom *et al* studied 42 patients with JIA and 41 controls and found that DR8 was associated with a higher aetiological fraction than DQ4, but the findings did not reach statistical significance.<sup>8</sup> However, in a similar study of a cohort of 43 DR8 positive patients with JIA and 24 controls van Kerckhove *et al* found the opposite, concluding that DQ4 is at least as likely a candidate for the primary HLA association in JIA.<sup>9</sup>

Another approach used is to analyse sequences of HLA class II alleles associated with JIA. Nepom *et al* noted that DRB1 alleles associated with pauciarticular JIA share a common motif.<sup>8</sup> The authors pointed out that the DRB1 alleles encoding DR3, DR5, DR6, and DR8 are identical at the positions encoding amino acids 40–53 and suggested that the DRB1 locus might be most relevant for the disease pathogenesis.<sup>8</sup> However, analysis of the sequences of all DQ alleles associated with early onset pauciarticular JIA showed that they are also similar. As pointed out by Haas *et al* the DQA1 alleles associated with JIA (that is, DQA1\*0401, 0501, 0601) share a sequence motif in positions 40–53.<sup>10</sup> Furthermore, all these alleles carry a mutation in the Y box of the promoter region.<sup>11</sup> Thus the approach based on the search for sequence motifs among HLA class II alleles associated with JIA has also failed to clarify whether DRB1\*08 or DQA1\*04 alleles are primarily predisposing for the disease.

The purpose of this study was to determine the primary association on the DR8-DQ4 haplotype—that is, whether JIA is primarily associated with DRB1\*08 or DQA1\*04. The frequency of rare DR8 haplotypes—that is, haplotypes which encode DR8 without DQ4, was analysed among a large cohort of patients ( $n=585$ ) and normal controls ( $n=3155$ ) from Norway. In addition, we studied a group of 47 patients with JIA and 158 controls from Poland, prompted by the observation that in the Polish population rare DR8 haplotypes may be more frequent.<sup>12</sup>

## PATIENTS AND METHODS

### Patients and controls

Five hundred and eighty five unrelated Norwegian patients and 47 Polish patients were studied. All patients met the American College of Rheumatology (formerly, the American Rheumatism Association) criteria for juvenile rheumatoid arthritis.<sup>13</sup> Only patients classified as having pauciarticular or polyarticular RF negative JIA were studied. Patients with polyarticular RF positive, systemic JIA and patients with spondyloarthropathies were excluded from the study. The Norwegian patients were divided into three groups (table 1). The first group (I) comprising 256 patients have been described previously.<sup>5</sup> The second group (II) included 221 patients with long term follow up (mean follow up period 15 years). The third group (III) comprised 108 patients who were followed

**Abbreviations:** JIA, juvenile idiopathic arthritis; LD, linkage disequilibrium; OR, odds ratio; RF, rheumatoid factor

**Table 1** Percentage of girls, average age at onset, and the frequencies of chronic iridocyclitis (CIC) and antinuclear antibodies (ANA) in patients with juvenile idiopathic arthritis (JIA)

JIA subset*	Number of patients	% Girls	Mean (SD) age at onset (months)	With CIC (%)	With ANA (%)
Group I (Norwegian):					
Pauciarticular JIA	207	71	65 (48)	21	35
RF negative polyarticular JIA	49	78	62 (43)	16	43
Group II (Norwegian):					
Pauciarticular JIA	160	73	87 (53)	21	41
RF negative polyarticular JIA	61	66	106 (48)	16	21
Group III (Norwegian):					
Pauciarticular JIA	73	75	61 (43)	14	37
RF negative polyarticular JIA	35	71	75 (50)	11	34
Polish JIA (pauciarticular)	47	81	29 (9.7)	36	38

\*Group I, Norwegian patients with JIA reported previously in reference 5; group II, Norwegian patients with JIA with 15 years follow up; group III, Norwegian patients with JIA with one year follow up.

up for more than one year. The Polish patients came from a cohort treated at the Institute of Rheumatology in Warsaw. All of them had pauciarticular JIA of more than 10 years' duration. The 3155 unrelated white subjects from the Norwegian Bone Marrow Donor Registry (NBMDR) were used as controls for the Norwegian patients with JIA. All these subjects were typed genomically for DRB1 alleles. Because they were not typed for DQ, we selected all 285 DRB1\*08 positive healthy controls and typed them for DQA1 and DQB1 alleles. As controls for the Polish patients, a group of 158 healthy unrelated subjects of Polish origin who had been previously typed genomically for DRB1 and DQ alleles were used.<sup>12</sup>

### Genomic HLA typing

The salting out method was used to prepare genomic DNA from peripheral blood leucocytes.<sup>14</sup> Polymerase chain reaction-sequence-specific oligonucleotide (PCR-SSO) typing was used to type patients and controls for the alleles of the DRB1, DQA1, and DQB1 loci as described previously.<sup>15</sup> When higher resolution was required a PCR-SSP kit was used (Dynal, Oslo, Norway).

Haplotypes were deduced based upon known patterns of LD.<sup>7</sup> The occurrence of a rare haplotype was postulated whenever a DR-DQ haplotype was found that did not correspond with frequent haplotypes.

### Nomenclature

Because data were generated using allele-specific oligonucleotide probes, which allow the most precise identification of the individual alleles, the recent form of the standard WHO nomenclature was used.<sup>16</sup> However, for simplicity, for the molecules we used serological nomenclature, where DR8 or DQ4 means DR( $\alpha$ ,  $\beta$ 1\*08) or DQ( $\alpha$ 1\*04,  $\beta$ 1\*04), respectively.<sup>16</sup>

### Statistical analysis

Odd ratios (OR) were calculated according to Woolf's method, and the statistical significance for differences between allele frequencies between patients and controls was calculated by the  $\chi^2$  test or Fisher's exact test when appropriate. In the analysis of the distribution of the HLA class II alleles among Norwegian patients, p values were not corrected for the number of comparisons because the associations found were all typical for JIA and they have been reported in previous studies of Norwegian patients with JIA. In the analysis of Polish patients p values were corrected for the number of alleles studied (n=13) according to the Bonferroni method. Stratified analysis of the data using Mantel-Haenszel test was performed using Epi-Info version 6 software.

### RESULTS

HLA-DRB1 typing showed that in the Norwegian cohort all groups had an increase in the frequency of DRB1\*0801 ( $p < 10^{-6}$ ) and a decrease in the frequency of DRB1\*0701 ( $p < 0.05$ ) among the patients with JIA compared with the controls. We also noted a decrease of DRB1\*04, both in pauciarticular ( $p < 10^{-6}$ ) and polyarticular JIA ( $p < 0.05$ ). Similarly, the Polish patients with pauciarticular JIA showed an increase in DRB1\*0801 (OR=11.6,  $p < 10^{-6}$ ) and a decrease of DRB1\*0701 (OR=0.2,  $p < 0.01$ ). There was also a trend towards a decrease of DRB1\*04. With respect to the DQA1 locus, the DQA1\*0401 allele was strongly associated with the disease.

An analysis of the DRB1-DQA1-DQB1 haplotypes showed that 225/585 (38%) Norwegian patients carried a DR8 haplotype, of whom 222 also carried DQ4. In the control group the respective numbers were 284/3155 (9%) and 282. In the Polish population 18/47 (38%) patients carried a DR8 haplotype, of whom 14 also carried DQ4. The numbers among the Polish controls were 8/158 (5%) and 6, respectively. To determine whether DRB1\*08 or DQA1\*04, or both, were primarily associated with the disease, we further analysed the DR8 haplotypes which were DRB1\*08 positive but which did not carry DQA1\*0401, called rare DR8 haplotypes. Table 2 gives the results obtained. We found such rare haplotypes among three Norwegian patients and two Norwegian controls. The frequency of haplotypes carrying DRB1\*08 without DQA1\*04 and DQB1\*04 was significantly higher among the Norwegian patients than among the controls (OR=8.1, 3/585 v 2/3155,  $p = 0.029$ ). The same association was observed in the Polish data set, where 4/47 patients compared with 2/158 (OR=7.3,  $p = 0.025$ ) controls carried haplotypes that were DRB1\*08 positive without carrying DQA1\*04 or DQB1\*04.

We also analysed the association of the rare DR8 haplotypes among patients with JIA and controls after exclusion of all DR8-DQ4 haplotypes. There was still a significantly higher frequency of rare DR8-non-DQ4 haplotypes in DR8-DQ4 negative patients than among the controls, both in the Norwegian (3/948 v 2/6028; OR=9.6;  $p = 0.02$ ) and the Polish cohort (4/79 v 2/310; OR=8.2;  $p = 0.017$ ). To evaluate the joint significance of our findings in both the Norwegian and Polish cohort we performed stratified analysis of the data using the Mantel-Haenszel test. We obtained highly significant results: OR=7.63 (95% CI 1.97 to 31.77),  $p < 0.0008$ .

Neither among Polish patients with JIA nor among the controls did we find any haplotypes which carried DQA1\*0401 and DQB1\*0402 in the absence of DRB1\*08. Similarly, in the Norwegian patients with JIA, we did not find any patient who was DQ4 positive and DR8 negative.

**Table 2** "Rare" DRB1\*08 encoding haplotypes among Norwegian and Polish patients and controls†

	DRB1	DQA1	DQB1	Diagnosis
Norwegian patients				
Group I (n=256)	<b>0801</b> -1501	0102- <b>0301</b>	<b>0302</b> -0602	Pauci-JIA
Group II (n=221)	<b>0801</b> -1101	<b>0102</b> -0501	0301- <b>0602</b>	Poly-JIA
Group III (n=108)	<b>0802</b> -1501	0103- <b>0104</b>	0501- <b>0601</b>	Poly-JIA
Norwegian patients pooled (n=585)	3/585 (0.5%)*			
Norwegian controls (n=3155)	<b>0801</b> -1301	0102- <b>0301</b>	<b>0302</b> -0604	
	0701- <b>0803</b>	<b>0103</b> -0201	0201- <b>0601</b>	
	2/3155 (0.06%)			
Polish patients (n=47)	0101- <b>0801</b>	0101- <b>0102</b>	0501- <b>0502</b>	Pauci-JIA
	<b>0801</b> -1301	<b>0102</b> -0103	<b>0602</b> -0603	Pauci-JIA
	0801- <b>0801</b>	<b>0102</b> -0401	0401- <b>0602</b>	Pauci-JIA
	<b>0801</b> -1301	0103- <b>0601</b>	<b>0301</b> -0603	Pauci-JIA
	4/47 (8.5%)**			
Polish controls (n=158)	<b>0801</b> -1501	0102- <b>0301</b>	<b>0302</b> -0602	
	0701- <b>0801</b>	0201- <b>0302</b>	0201- <b>0302</b>	
	2/158 (1.3%)			

†Haplotypes which were DRB1\*08 positive but which did not carry DQA1\*0401, called "rare" DR8 haplotypes, are indicated in bold; \*p=0.029 (3/585 v 2/3155) comparing patients and controls; \*\*p=0.025 (4/47 v 2/158) comparing patients and controls.

## DISCUSSION

The distribution of HLA-DRB1-DQA1-DQB1 haplotypes among patients with JIA and healthy controls has been studied among two populations: Norwegian and Polish. As expected, we found a strong association with the DR8-DQ4 (that is DRB1\*08-DQA1\*04-DQB1\*04) haplotype in both groups. Interestingly, we also found that a significantly higher percentage of patients than controls carried haplotypes encoding DR8 without DQ4. These results suggest that on the DR8-DQ4 haplotype the DRB1\*08 allele may be primarily associated with susceptibility to develop JIA, whereas the association with DQA1\*04 and DQB1\*04 is caused by LD to DRB1\*08.

The finding that DRB1\*08 confers a higher risk for development of JIA is consistent with the results of Nepom *et al* who found that among the patients with JIA the frequency of DR8 was higher than DQ4, although the results in that study were not statistically significant.<sup>8</sup> Apart from the association with DRB1\*08 and DQA1\*04, JIA is also associated with other HLA class II alleles, including a number of other DQA1 and DQB1 alleles. Thus it is important to consider whether the rare DR8 haplotypes we have found, might predispose to the development of the disease through one or more of the other DQA1 or DQB1 alleles carried by the patients. However, this does not seem to be the case. Firstly, the DQA1\*0301, DQB1\*0302 alleles (found in one patient and three controls) are normally found on the DR4-DQ8 haplotype, which is regarded as a protective haplotype in seronegative JIA (table 2) (although it is strongly associated with predisposition to rheumatoid arthritis and seropositive JIA).<sup>5</sup> Secondly, all but one of the remaining rare DR8 haplotypes found among patients in the Polish and Norwegian population carried the DQA1\*0102 allele. This allele is usually found on the DR15 and DR16 haplotypes, which have been described as not being associated with JIA.<sup>17</sup> Only one of the haplotypes classified by us as rare carried the DQA1\*0601 and DQB1\*0301 alleles, which have previously been suggested to be positively associated with JIA.<sup>10</sup> One of the analysed Norwegian patients with JIA who carried the rare DR8 haplotype also carried DRB1\*11, an allele known to be associated with pauciarticular JIA.<sup>4</sup> However, in our investigated cohort we did not find any significant association with the DRB1\*11 allele, either in the Norwegian or the Polish population. Similarly, two of the Polish patients who carried the rare DR8 haplotype also carried the DRB1\*13 allele, which did not show significant association with JIA in the Polish population.

Only DRB1\*08 positive Norwegian controls were typed for DQ, hence the frequency of DRB1\*08 negative -DQA1\*04 positive haplotypes among the controls could not be determined. However, because we did not find any such haplotypes among the Norwegian patients, if they should occur among the controls this would only strengthen our conclusions. In the Polish population we did not find any haplotype which carried DQA1\*04 and DQB1\*0402 in the absence of DRB1\*08, either among patients or controls.

This study is also the first demonstration that JIA in the Polish population has similar HLA class II associations as those found among white subjects of Western Europe and North America, particularly the strong association with DRB1\*08.

In conclusion, our study of cohorts of patients with JIA from two populations strongly suggests that the association with the DRB1\*08 allele is primary and responsible for the observed association with the DR8-DQ4 haplotype.

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## ECHO

## Recognising macrophage activation syndrome



Please visit the *Annals of the Rheumatic Diseases* website [www.annrheumdis.com] for link to this full article.

A profound drop in platelets indicates macrophage activation syndrome (MAS) in rheumatic disease in children. This is just one observation by Sawhney *et al* to emerge from a retrospective review over 20 years in a tertiary paediatric rheumatology unit.

The review of nine children—the largest reported series so far—presents valuable information on this rare, poorly understood, and potentially fatal complication of systemic onset juvenile idiopathic arthritis (SOJIA) and other forms of juvenile idiopathic arthritis. Better understanding is vital for correct early diagnosis and treatment.

Seven children had SOJIA, one enthesitis related arthritis, and one chronic infantile neurological cutaneous articular syndrome. Mean duration before MAS was about four years, but it also occurred at presentation of the rheumatic disease. Characteristic features were intense, persistent fever, new onset enlarged liver and spleen, and swollen lymph glands; an early drastic drop in platelet count; and drops of varying extent in white blood cells and haemoglobin. Phagocytosis of platelets by macrophages in bone marrow confirmed the diagnosis in four of seven children. Abnormal liver function was also observed. Treatment was with high dose steroids in all children plus other immunosuppressants in some; two children with renal disease died.

A preceding infection occurred in most children. The authors found no trigger for MAS among the treatments received at or before its onset. As they describe, MAS in rheumatic disease has special features which help to distinguish it from other macrophage disorders and the flare ups characteristic of SOJIA—essential to avoid delayed diagnosis.

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