HLA Associations in a Cohort of Children With Juvenile Idiopathic Arthritis With and Without Uveitis

Sheila T. Angeles-Han,1–3 Courtney McCracken,1 Steven Yeh,3 Se Ryeong Jang,1 Kirsten Jenkins,2 Spencer Cope,4 John Bohnsack,4 Aimee Hersh,4 Susan D. Thompson,5 and Sampath Prahalad1,2

1Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia, United States
2Children’s Healthcare of Atlanta, Atlanta, Georgia, United States
3Department of Ophthalmology, Emory University School of Medicine, Atlanta, Georgia, United States
4Department of Pediatrics, University of Utah, Salt Lake City, Utah, United States
5Department of Pediatrics, Cincinnati Children’s Medical Center, Cincinnati, Ohio, United States

Correspondence: Sheila T. Angeles-Han, Division of Pediatric Rheumatology, Emory University, 2015 Uppergate Drive, Atlanta, GA 30322, USA; sangle@emory.edu.
Submitted: April 24, 2015
Accepted: August 9, 2015
Citation: Angeles-Han ST, McCracken C, Yeh S, et al. HLA associations in a cohort of children with juvenile idiopathic arthritis with and without uveitis. Invest Ophthalmol Vis Sci. 2015;56:6043–6048. DOI:10.1167/iovs.15-17168

PURPOSE. Juvenile idiopathic arthritis (JIA)–associated uveitis can lead to ocular complications and vision loss. Alleles HLA-DRB1*08, *11, and *13 are risk alleles for JIA, whereas HLA-DRB1*11 and *13 alleles increase uveitis susceptibility. We examined the association of common HLA-DRB1 alleles in children with JIA alone and JIA-associated uveitis.

METHODS. High-resolution HLA-DRB1 genotyping was performed in 107 children with oligoarticular and polyarticular rheumatoid factor (RF) negative JIA and 373 non-Hispanic white controls. Children with JIA alone and JIA-associated uveitis were of similar race, ethnicity, sex, and age at arthritis diagnosis. Odds ratios (OR) and 95% confidence intervals (CI) were calculated.

RESULTS. There were 47 children with JIA-associated uveitis and 60 with JIA alone. Compared to controls, only children with JIA-associated uveitis had increased odds of carriage of HLA-DRB1*11 (OR, 2.2 95% [CI, 1.1–4.3], P = 0.025). There also was increased carriage of HLA-DRB1*08 and *13 (OR, 12.6 [95% CI, 2.0–77.8], P = 0.011). Compared to controls and children with JIA alone, those with JIA-associated uveitis had increased odds of carriage of HLA-DRB1*11 and *13 (OR, 9 [95% CI, 2.8–29.0], P < 0.0001 and OR, 8.6 [95% CI, 1.0–74.4], P = 0.042), respectively.

CONCLUSIONS. We report the novel finding that carriage of HLA-DRB1*11 and *13 appears to increase the risk of uveitis in children with JIA.

Keywords: uveitis, HLA, juvenile idiopathic arthritis

Uveitis is the most common extraarticular manifestation of juvenile idiopathic arthritis (JIA) and can increase a child’s risk for ocular complications and permanent vision loss. Juvenile idiopathic arthritis is a chronic childhood arthritis of unknown etiology consisting of 7 categories that differ in their risk for uveitis development.1 Children with oligoarticular and polyarticular rheumatoid factor (RF)-negative JIA are considered at highest risk. Clinical risk factors for uveitis are well-established, but less extensive investigation has been conducted on genetic associations.

Studies in JIA-associated uveitis have focused on HLA-DRB1 alleles in children with oligoarticular and polyarticular RF-negative JIA because of their increased risk for uveitis development. In JIA, associations have been confirmed with HLA and non-HLA genetic variants.2,3 These include HLA Class II genes, such as HLA-DRB1*13:01;DQA1*01:03;DQB1*06:03 in oligoarticular JIA, and DRB1*08:01;DQA1*01:01;DQB1*04:02 and DRB1*11:03;1104;DQA1*05:01;DQB1*03:01 haplotypes in polyarticular RF negative JIA.4 There are also reports of increased arthritis risk in adults with rheumatoid arthritis and in children with RF-positive JIA with certain HLA genetic combinations, such as HLA-DRB1*04:01/04:04.5,6 Principal components analysis of HLA class I and II loci has shown the clustering of young onset oligoarticular and RF-negative polyarticular JIA patients.4 These children are at greatest risk for uveitis development, hence the focus of our study.

Our objective was to explore the association of known HLA-DRB1 alleles in a cohort of children with oligoarticular and polyarticular JIA who develop uveitis. We also investigated whether there is a synergistic effect of risk variants that predispose to the development of two autoimmune diseases – arthritis and uveitis. We sought to determine if children with JIA-associated uveitis demonstrate differences in HLA-DRB1 alleles or allele combinations compared to children with JIA alone. Recently, specific and shared genetic associations were noted in individuals with acute anterior uveitis and ankylosing spondylitis, suggesting distinct genetic profiles in individuals with arthritis who develop uveitis.7 Although earlier studies used old terminology when reporting HLA data, for uniformity, we will attempt to use the new nomenclature in our study and discussion when possible. However, we are not always able to determine whether HLA-DR5 refers to DRB1*11 or DRB1*12.

Patients and Methods

Subjects
We enrolled 107 non-Hispanic white (NHW) children with oligoarticular or polyarticular RF negative JIA (60 with JIA alone...
and 47 with JIA-associated uveitis). We only included children who were diagnosed with JIA for at least 4 years since >80% of children who have uveitis do so within that timeframe.\(^8,9\) There were no statistically significant differences in JIA category, sex, race, ethnicity, or age at arthritis diagnosis between subjects with and without uveitis (data not shown). The healthy control group consisted of 373 healthy NHW individuals from the University of Utah and Cincinnati Children's Hospital. Some of the controls were included in a previous study.\(^4\) Approval from the institutional review boards at each institution was obtained. We followed the tenets of the Declaration of Helsinki and informed consent was obtained after we explained the nature of the study.

**HLA-DRB1 Genotyping and Classification**

We extracted DNA from peripheral blood mononuclear cells per established protocols and performed high-resolution sequence-based HLA-DRB1 genotyping on all patients and controls. We only considered HLA-DRB1*08, *11, and *13 as risk alleles as these are strongly associated with JIA and have been reported in JIA-associated uveitis. Our analysis was limited to two digit HLA resolution because of the modest sample size.

**Statistical Analysis**

Statistical analysis was conducted using SAS v 9.3 (SAS Institute, Inc., Cary, NC, USA). Statistical significance was assessed at the 0.05 level unless otherwise noted. Descriptive statistics were calculated for all variables of interest and include means and standard deviations or counts and percentages when appropriate. We compared the frequencies of HLA-DRB1 alleles between children with JIA alone and control subjects, children with JIA-associated uveitis and control subjects, and children with JIA alone and JIA-associated uveitis using \(\chi^2\) or Fisher's exact tests as appropriate. Odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated. We compared children with JIA-associated uveitis to controls to determine if there were initial differences in HLA risk alleles before comparing to children with JIA alone. Likewise, although most children with JIA will suffer uveitis within the first 4 years of their JIA diagnosis, we were unable to account for delayed uveitis onset and subclinical disease.

To control for multiple comparisons, we applied a Bonferroni correction using an adjusted significance level of 0.05/9 = 0.006. This adjustment was chosen because each primary HLA allele configuration (HLA-DRB1*08, HLA-DRB1*11, HLA-DRB1*13) was tested three times and there were three planned pairwise comparisons for each configuration (JIA alone versus control, JIA-associated uveitis versus control, and JIA alone versus JIA-associated uveitis).

**RESULTS**

There were 107 NHW children with oligoarticular or polyarticular RF-negative JIA. Median age at JIA diagnosis was 3.5 years (2.2–5.5) for those with JIA and 3.1 years (2.0–4.0) for those with JIA-associated uveitis. Approximately 75% of children had oligoarticular JIA and 25% had polyarticular RF-negative JIA. Of children with JIA alone 12% (7/60) were ANA positive versus 62% (29/47) with JIA-associated uveitis, which was significantly different (\(P < 0.0001\)). In children with uveitis, the median age at uveitis diagnosis was 4.0 years (interquartile range [IQR] 2.8–5.6) with a median duration of arthritis before uveitis of 1.3 years (IQR 0.1–3.5). Most had bilateral disease (79%) and several ocular complications.

We first examined carriage of single risk alleles. On examining HLA-DRB1 alleles in 60 children with JIA alone and 573 control subjects, we confirmed a significant association between JIA and HLA-DRB1*08 (23.3% JIA alone versus 5.1% controls; OR, 5.6 [95% CI, 2.7–12.1], \(P < 0.0001\)) and DRB1*13 (36.7% JIA alone versus 23.1% controls; OR, 1.9 [95% CI, 1.1–3.4], \(P = 0.023\); Table 1). On examining HLA-DRB1 alleles in 47 children with JIA-associated uveitis and 373 controls, significant associations were noted with HLA-
<table>
<thead>
<tr>
<th>Author</th>
<th>JIA-Associated Uveitis, N</th>
<th>JIA Sub-Type</th>
<th>JIA Alone, N</th>
<th>Controls, N</th>
<th>HLA-DR Association*</th>
<th>Other Associations*</th>
<th>Genetic Combinations*</th>
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<tbody>
<tr>
<td>Glass et al.18</td>
<td>24</td>
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<td>HLA-DRw5 and ANA†</td>
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<td>38</td>
<td>Oligoarticular</td>
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<td>HLA-DR5 RR: 3.52‡</td>
<td>HLA-A2, HLA-Bw44, HLA-Bw16, HLA-Bw35, HLA-Cw4, MT2</td>
<td>HLA-Bw44, HLA-Cw4, HLA-DR5 RR 20.77‡</td>
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<td>70</td>
<td>Oligoarticular</td>
<td>91</td>
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<td>HLA-DR5 OR 3.51</td>
<td>HLA-DR1 OR 0.19</td>
<td>HLA-DPw2 I</td>
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<td>69</td>
<td>218</td>
<td>HLA-DRB1*11:04 OR 3.45† HLA-DR1 OR 0.18†</td>
<td>HLA-DQAI*01:01 Protective</td>
<td>HLA-DRB1<em>11:04 and HLA-DPB1</em>02:01 OR 7.77**</td>
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<td>HLA-DR5 and HLA-DRw8 NS†</td>
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<td>131</td>
<td>181</td>
<td>HLA-DRB1*01:01 Protective</td>
<td>HLA-DQAI<em>01:01, HLA-DQB1</em>05:01 Protective</td>
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<td>74</td>
<td>192</td>
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<td>HLA-A2, HLA-Bw62 ANA and HLA-DR5 ANA and HLA-A2</td>
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<td>186</td>
<td></td>
<td>HLA-DR1 OR 0.27†</td>
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<td>HLA-A19, HLA-B22, HLA-DR2</td>
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<td>99</td>
<td>228</td>
<td>HLA-DRB1*13 OR 3.4 in replication</td>
<td>HLA-A, HLA-B, or SNPs for TNFalpha, HLA-E and DIF-2 NS†</td>
<td>230 healthy parents of cases 130 healthy parents of cases</td>
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TABLE 2. Continued

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<th>Author</th>
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<th>JIA Alone, N</th>
<th>Controls, N</th>
<th>HLA DRDR1 Association</th>
<th>Other Associationsa</th>
<th>Genetic Combinationsb</th>
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<tr>
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<td>HLA-DRB1*11/13</td>
<td>HLA-DRB1<em>08/11, HLA-DRB1</em>08/13</td>
<td>HLA-DRB1*11/13/08</td>
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<td></td>
<td>OR 3.47, 95% CI 1.16–7.47, p = 0.001</td>
<td>HLA-DRB1<em>11/13, HLA-DRB1</em>08/11</td>
<td>HLA-DRB1*11/13/08</td>
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Other Associationsa

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<th>HLA-DRB1*11/13</th>
<th>HLA-DRB1<em>08/11, HLA-DRB1</em>08/13</th>
<th>HLA-DRB1*11/13/08</th>
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<tbody>
<tr>
<td>OR 0.26, 95% CI 0.12–0.57, p = 0.001</td>
<td>HLA-DRB1<em>11/13, HLA-DRB1</em>08/11</td>
<td>HLA-DRB1*11/13/08</td>
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</table>

a Associations reported in terminology used in their respective studies.
b† Compared to JIA alone.
‡ Compared to controls.

* Associations reported in terminology used in their respective studies.
† Compared to JIA.‡ Compared to controls.

We next determined if the risk of JIA-associated uveitis was increased in children who carried two risk alleles. Of note, we did not find any children who were homozygous for HLA-DRB1*08, *11, or *13. Carriage of two risk HLA DRB1 alleles was not significantly different between children with JIA alone compared to healthy controls. However, carriage of two risk HLA DRB1 alleles was significantly higher among children with JIA-associated uveitis compared to JIA alone (6.3% JIA-associated uveitis versus 1.6% JIA alone; OR, 3.47 [95% CI, 1.16–7.47]; P = 0.001). When comparing children with JIA-associated uveitis and JIA alone, we again noted a significant association with HLA-DRB1*11 and DRB1*13 (12.8% JIA-associated uveitis compared to healthy controls: (1) HLA-DRB1*11 and DRB1*13 (12.8% JIA-associated uveitis versus 1.6% controls; OR, 9 [95% CI, 2.8–29.0]; P < 0.0001) and (2) HLA-DRB1*08 and DRB1*13 (6.4% JIA-associated uveitis versus 0.5% controls; OR, 12.6 [95% CI, 2.0–77.8], P = 0.011).

After adjusting our significance level to 0.006 to control for multiple comparisons, an increased odds of JIA-associated uveitis remained statistically significant for HLA-DRB1*11/13 compared to controls. However, compared to JIA alone, the clinically significant increased odds of JIA-associated uveitis observed for HLA-DRB1*11/13 (OR = 8.6) was no longer statistically significant. This also was true when comparing the frequency of HLA-DRB1*11, HLA-DRB1*13, and HLA-DRB1*08/13 among JIA-associated uveitis and control patients, and when comparing frequency of HLA-DRB1*13 among JIA-alone and controls. HLA-DRB1*08 remained statistically significant for JIA alone and JIA-associated uveitis compared to controls.

**DISCUSSION**

Alleles HLA-DRB1 have been associated with JIA, and HLA-DRB1*11 and *13 alleles increase susceptibility to uveitis. We confirm associations between JIA, JIA-associated uveitis and HLA-DRB1*08, *11, and *13. We newly describe, to our knowledge, an association between uveitis and carriage of two HLA-DRB1 risk alleles, HLA-DRB1*11/13 or *08/*13, compared to children with JIA alone, suggesting a possible gene dosage effect. Our findings are novel given that we describe the combination of two risk alleles in a population of children with JIA-associated uveitis compared to JIA alone and controls, whose size is comparable to earlier studies.

As reviewed elsewhere, several HLA associations have been confirmed in chronic infectious uveitis, such as in acute anterior nongranulomatous uveitis (HLA-B27), Vogt-Koyanagi-Harada’s disease (HLA-DRB1*04), Birdshot retinochoroidopathy (HLA-A29), and intermediate uveitis (HLA-DR2, HLA-DR15, HLA-B8, and HLA-B51). Distinct associations were noted recently in adults with acute anterior uveitis with and without ankylosing spondylitis.7 However, less extensive work has been conducted in children with JIA-associated uveitis. Family and sibling studies have supported a genetic association, but not an increased sibling recurrence ratio in...
uveitis.11–14 Most investigations to date focus primarily on the HLA region.15

An approximately 3-fold risk for uveitis has been attributed to HLA-DRB5*16–20 (which subsequently has been split into DRB1*11/13 and DRB1*12), and a protective effect of HLA-DRB1*15.47–50 Examination of DRB1*11 and DRB1*12 individually in children with JIA and JIA-associated uveitis has shown an increased risk for uveitis in HLA-DRB1*11/04 in linkage equilibrium with HLA-DOQA1*05:01 and HLA-DOQA1*03:01.20,22 Our study confirmed the association of uveitis with HLA-DRB1*11 and *13 in children with JIA.

Multiple risk alleles have been reported to influence phenotype, particularly related to early JIA susceptibility.20 It is unclear whether confirmed HLA variants predispose to arthritis and uveitis separately, or if there are synergistic effects related to eye disease. Our data suggest a potential gene dosage effect in children with JIA-associated uveitis when compared to those with JIA alone and controls. This was similar to a study of 15 children with JIA who carried DRB1*11:04 and DPB1*02:01 alleles, wherein 11 also had uveitis with an increased odds of 7.8-fold.20 Similarly, 8 of 18 children with JIA-associated uveitis and HLA-DRB1*11/04 additionally had either HLA-DRB1*11:04 or HLA-DRB1*13:01. This combination was not found in 12 controls. Haas et al.25 noted that children with uveitis had three associated alleles, specifically, the HLA-DRB1 allele on haplotypes and DPB1*02:01 (RR 3.11), compared to those without uveitis.25 Another group noted that, 5 years after JIA onset, children with (1) HLA-DR5 and HLA-DP2.1 positivity but HLA-DRB1*01 negativity, or (2) HLA-DR5 and HLA-DRB1*08 positivity had a cumulative risk for uveitis of approximately 75%.19 We show that when comparing children with JIA-associated uveitis to those with JIA alone or to controls, a combination of multiple HLA-DRB1 alleles may predispose to uveitis and, hence, influence phenotype. Further elucidation on the complex pathogenesis of JIA-associated uveitis, specifically with regards to the genetic predisposition is important.

There were several limitations to our study. Due to our small sample size, we were unable to conduct tests of heterogeneity to determine strengths of associations compared to controls. We limited our chosen loci to those previously reported in the literature to validate in our cohort. We felt that given our small numbers, two digit resolution was appropriate, and was also similar to previous reports. However, despite our small cohort, our finding that the combination of the carriage of HLA-DRB1*11 and DRB1*13 was rare as it was only observed in 6/373 controls and 1 child with JIA compared to 6/47 children with JIA-associated uveitis.

Our controls were enrolled in Utah and Cincinnati, and our cases were enrolled in Utah and Atlanta. However, our controls have been genotyped using different platforms in previous studies and have not demonstrated population stratification. This could be a potential limitation.37

In summary, our preliminary work shows that children with JIA and JIA-associated uveitis share HLA-DRB1-associated alleles, but children with certain combinations of risk alleles may have a higher predilection for developing uveitis. We newly showed, to our knowledge, that combinations of HLA-DRB1 genes in children with JIA may predispose to uveitis development. Understanding the genetic risk for uveitis may lead to improvements in the screening algorithm for patients with JIA.

Acknowledgments
The authors thank Mina Rohani-Pichavant for technical assistance. Supported by the National Eye Institute (NEI; Bethesda, MD, USA) K25EY021760 (STA); in part by the Cincinnati Children’s Research Foundation and its Cincinnati Genomic Control Cohort; by the Emory-Children’s Pediatric Research Center Biostatistics Core; and by the National Institute of Arthritis and Musculoskeletal and Skin Diseases (R01-AR060893). The Marcus Foundation, Inc., and the Arthritis Foundation (SP). Use of the Cincinnati Genomic Control Cohort was supported in part by the Cincinnati Children’s Research Foundation. The authors alone are responsible for the content and writing of this paper.

Disclosure: S.T. Angeles-Han, None; C. McCracken, None; S. Yeh, None; S.R. Jang, None; K. Jenkins, None; S. Cope, None; J. Bohnsack, None; A. Hersh, None; S.D. Thompson, None; S. Prahala, None

References


