Article type : Full Length

An amino acid motif in HLA-DRβ1 distinguishes patients with uveitis in juvenile idiopathic arthritis

Short title (running header): YST-motif in HLA-DRβ1 associated with JIA-uveitis

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Funding statement: This study was funded by: The Dr. F.P. Fischer Stichting, Amersfoort; The ODAS Stichting, the Landelijke Stichting Voor Blinden en Slechtzienden, Utrecht; the Stichting Nederlands Oogheelkundig Onderzoek (SNOO), Rotterdam, the Netherlands. Funding for the ICON-JIA study group: Research grant of the Federal Ministry of Education and Research [FKZ 01ER0812, FKZ 01ER0813; FKZ 01ER0828]. SLP is funded in part by the Li Ka Shing Foundation.

Disclosure statement: The authors have declared no conflicts of interest.

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Abstract

Objectives

Uveitis is a visually-debilitating disorder that affects up to 30% of children with the most common forms of juvenile idiopathic arthritis (JIA). The disease mechanisms predisposing only a subgroup of children to uveitis are unknown. To identify genetic susceptibility loci for uveitis in JIA, we conducted a genome-wide association study totalling 522 JIA cases.

Methods

We genotyped two cohorts of JIA patients with ophthalmological follow-up and then imputed the data using a genome-wide imputation reference panel, and an HLA-specific reference panel used for imputing amino acids and HLA types in the major histocompatibility complex (MHC). After imputation, we performed genome-wide and MHC-specific analyses, and used a reverse immunology approach to model antigen presentation at 13 common HLA-DRβ1 alleles.

Results

We identified the amino acid serine at position 11 (serine-11) in *HLA-DRβ1* as associated to increased risk of uveitis (OR = 2.60, $p = 5.43 \times 10^{-10}$) and specific to females ($p_{females} = 7.61 \times 10^{-10}$, $p_{males} = 0.18$). Serine-11 resides in the YST-motif in the peptide binding groove of HLA-DRβ1; all three amino acids are in perfect linkage disequilibrium and show identical association to disease.

Quantitative prediction of binding affinity revealed that discernable peptide-binding preferences distinguish HLA-DRβ1 alleles with the YST-motif.

Conclusion

Our findings highlight a genetically distinct, sexually-dimorphic feature of JIA-uveitis compared to non-uveitis JIA. The association indicates the potential involvement for antigen presentation by HLA-DRβ1 in the development of uveitis in JIA. This work will advance our progress towards treating and preventing sight-threatening complications of uveitis in children with JIA.

Introduction

Juvenile idiopathic arthritis (JIA) is the most common chronic rheumatic disease in childhood, affecting approximately 16-150 per 100,000 individuals (1). As many as 1 in 3 children that suffer from the most common JIA categories, oligoarticular and polyarticular rheumatoid factor-negative JIA, develop chronic anterior uveitis. Uveitis in JIA specifically is a chronic inflammatory eye disease, and the most frequent extra-articular manifestation of JIA (2). Uveitis threatens the sight of those affected, and can result in complications including band keratopathy, cataracts, glaucoma, macular edema, and, in severe cases, hypotony (2). Because it typically afflicts those younger than seven years old, uveitis can dramatically impact the quality of life of children and can continue to impact patients into adulthood (2,3).

Early detection and adequate ophthalmological management of JIA-associated uveitis (i.e., JIA-uveitis) are critical to prevent sight-threatening complications (4,5). Despite its severity, chronic anterior uveitis is typically insidious in onset and often becomes symptomatic only after irreversible damage has occurred. Consequently, rigorous ophthalmological screening of all JIA patients is required for early detection and prompt treatment of uveitis associated with JIA (6). Despite screening, severe ocular complications may already be present at the time of a uveitis diagnosis.

Both JIA and uveitis are complex, multifactorial autoimmune disorders with both genetic and environmental risk factors (2,3,7). Genome-wide association studies (GWAS) in JIA (regardless of uveitis status) have revealed a number of loci associated to the disease (8–10) which collectively explain ~20% of the phenotypic variation (8). A number of HLA alleles have also been identified as increasing risk of JIA-uveitis compared to controls (11). However, genome-wide genetic markers that distinguish JIA without uveitis from JIA-uveitis remain elusive. Further, while females comprise the majority of JIA patients (female-male ratio ~2:1) (12), the extent to which biological risk for JIA-uveitis is sexually dimorphic is unknown (12).

Here, we performed genotyping and imputation of the major histocompatibility complex (MHC) in 192 JIA-uveitis cases and 330 JIA patients without uveitis, with the aim of identifying those genetic variants that segregate more commonly in JIA patients with uveitis compared to those without uveitis.

Results

We performed data collection, quality control and analysis in two phases that we then jointly analyzed to improve power for locus discovery (**Table 1,** and **Supplementary Information**) (13). Samples were collected from: (a) a Dutch cohort (here called Phase 1), and (b) a cohort of samples collected in Germany, Belgium and Switzerland (here called Phase 2). We genotyped all samples on the Infinium HumanOmniExpress-24v1.1 array and in each phase, performed sample- and variant-level quality control (QC), prephasing, and imputation (**Methods** and **Supplementary Information**).

Genome-wide association testing

Our data enabled three comparisons: (a) JIA cases vs. population-level controls, (b) JIA-uveitis cases vs. population-level controls, and (c) JIA-uveitis cases vs. non-uveitis JIA. As we sought to compare the genetic architectures of JIA and uveitis, and because far larger GWAS of JIA and population-level controls have already been completed (14), we performed the first two comparisons for QC and to look up known associations (14,15) (**Supplementary Figures 1** and **2,** and **Supplementary Table 1**). We account for these comparisons when considering multiple testing, and focus here on the comparison of JIA-associated uveitis cases vs. non-uveitis JIA samples.

We performed a GWAS within each study phase, correcting for sex and the first two principal components (PCs) (**Methods**, **Supplementary Figures 3** and **4**).

We meta-analyzed the results using METAL (16) for a combined analysis of 330 non-uveitis JIA samples and 192 uveitis cases (**Table 1**). The single signal achieving genome-wide significance ($p < 2 \times 10^{-8}$, accounting for three phenotype comparisons) resided in the MHC (**Supplementary Figures 3** and **4**).

Association testing and fine-mapping in the MHC

To identify the amino acids or HLA types driving the genome-wide association signal, we imputed the MHC region on chromosome 6 using an MHC-specific imputation panel (17). We merged the imputation dosages from Phases 1 and 2 to perform a mega-analysis, running a logistic regression across the merged data and correcting for the top 5 PCs, sex, and analysis phase (i.e., Phase 1 or Phase 2). This 'mega-analysis' approach is theoretically and empirically highly similar to inverse variance-weighted meta-analysis (18,19) (confirmed in our own data; **Methods** and **Supplementary Figure 5**) and has the additional advantage of allowing for interaction testing and conditional analysis.

Of the SNPs, amino acids, and classical alleles tested in the MHC (**Supplementary Table 2**), we observed the strongest association for the presence of either serine or aspartic acid at position 11 in *HLA-DRB1* (OR = 2.59 [95% CI: 1.92 - 3.50], $p = 4.80 \times 10^{-10}$; **Figure 1** and **Table 2**). To identify which of the possible residues at position 11 explained the signal, we first performed an omnibus test of all but one of the 6 alleles present at *HLA-DRB1* position 11 compared to a null model. We found that the goodness-of-fit of the

omnibus test far exceed that of a null model including only sex, PCs and study phase (likelihood ratio test $p = 1.5 \times 10^{-9}$).

Next, to test if a single *HLA-DRB1* position 11 allele was driving the top association signal (as opposed to all possible alleles together, as modeled by the omnibus test), we performed conditional testing of the top association signal by first including the imputation dosages for serine and then separately for aspartic acid at position 11 as an additional covariate in the logistic regression model (**Figure 1** and **Table 2**). After conditioning on serine-11, the association signal dropped substantially ($p = 0.069$), while conditioning on aspartic acid at position 11 left the signal essentially unchanged (OR = 2.60 [95% CI: 1.92 - 3.52], $p = 5.43 \times 10^{-10}$), indicating that serine explains the bulk of the signal. The association for presence of serine at position 11 in *HLA-DRB1* was consistent in both Phase 1 (OR = 2.15 [95% CI: 1.52 - 3.05], $p = 1.59 \times 10^{-5}$) and Phase 2 (OR = 3.34 [95% CI: 1.92 - 5.83], $p = 2.11 \times 10^{-5}$), indicating that both study phases contributed to the overall mega-analysis signal (13). Subsetting the sample to those with oligo-articular (extended or persistent) or rheumatoid factor-negative polyarticular JIA, the phenotypes in which typical chronic anterior uveitis most commonly occurs, did not affect the association results (serine-11 OR = 2.42, $p = 1.72 \times 10^{-8}$; **Supplementary Information**).

HLA-DRB1 serine-11 is positioned in the middle of what is known as the YST-motif (20,21); the motif is comprised of tyrosine (Y) at position 10, serine (S) at position 11, and threonine (T) at position 12. All three amino acids are in perfect linkage disequilibrium (LD) with one another (i.e., $r^2 = 1$ between all residue

pairs). Additionally, the residues in the YST-motif is in near-perfect LD (r^2 = 0.9999994) with a fourth residue, presence of serine or glycine at position 13 (OR = 2.47 [95% CI: 1.84 - 3.32, p = 1.44 x 10^{-9}). Thus, all four amino acid configurations show statistically identical association to JIA-uveitis (**Table 2**). To try and further decipher the specific residue(s) across positions 10-13 driving the association in uveitis, we performed a series of likelihood ratio tests. In brief, a likelihood ratio test compares the fit of two models to the observed data and indicates if either model best fits that data. First, as expected, we found that a model containing serine-11 (or tyrosine-10 or threonine-12) best fit the data (p = 1.48 x 10^{-10}, compared to a model containing only PCs and sex), consistent with our initial association testing. Next, we combined the serine-or-glycine residue at position 13 with the YST-motif in a single model, and compared that model to one containing PCs, sex, and serine-11. The model including serine/glycine-13 only modestly improved the model fit (likelihood ratio test p = 0.043); neither serine-13 nor glycine-13 alone improved the model (p = 0.70 for both tests).

Next, we wanted to test whether the serine-11 uveitis signal was independent from previous associations in all JIA (including uveitis cases) found at *HLA-DRB1* serine-13 and glycine-13 (14) and replicated in our own data (**Supplementary Table 3**). We observed modest LD between serine-11 and serine-13 (r^2 = 0.621) and serine-11 and glycine-13 (r^2 = 0.104). Conditioning serine-11 on serine-13 in our JIA-uveitis vs non-uveitis JIA analysis, we found the serine-11 signal to be modestly affected (OR = 2.61 [95% CI: 1.74 - 3.93], p = 3.83 x 10^{-6}). Similarly, conditioning on glycine-13 revealed a modest signal change (OR =

2.41 [95% CI: 1.75 - 3.32], p = 6.49 x 10^{-8}). These results indicate that the association signal at serine-11 represents a primarily independent effect from that of serine-13 and glycine-13 in all JIA.

Sexual dimorphism in JIA-uveitis

As uveitis is epidemiologically known to be more prevalent in females with oligoarticular JIA (22), we wanted to formally test if the genetic association signal in uveitis was sexually dimorphic. In analyzing females only (136 JIA-uveitis samples vs. 228 non-uveitis JIA samples), we found a genome-wide significant signal at serine-11 in *HLA-DRB1* (OR = 3.30 [95% CI: 2.26 - 5.83], p = 7.61 x 10^{-10}); we found no evidence for association in males (56 JIA-uveitis cases vs. 102 JIA non-uveitis samples; OR = 1.41 [95% CI: 0.86 - 2.31], p = 0.177; **Table 2** and **Supplementary Figure 6**). Although the most significant association with uveitis in females was mapped to amino acid position 233 in the cytoplasmic domain of *HLA-DRB1* (presence of threonine, OR = 0.30 [95% CI: 0.20 - 0.41], $p = 3.50 \times 10^{-10}$; **Table 2**), this position is in near-perfect LD (r^2 = 0.98) with the residues in the YST-motif (positions 10-12), as well as presence of serine or glycine at position 13 (**Supplementary Figure 7**), and consequently yields a nearly identical association to that at serine-11 (**Table 2**).

To further explore the potential for a sex-specific effect, we ran the association test at serine-11 across all samples and included an interaction term between sex and the imputed dosage for presence of serine-11. We found a significant effect of the interaction term (OR = 2.25 [95% CI: 1.22-4.15], p = 0.0096),

indicating that the *HLA-DRB1* serine-11 signal was indeed sex specific. Reducing our analysis to only oligoarticular (extended or persistent) and RF-negative JIA left our interaction results unchanged ($p = 0.0121$; **Supplementary Information**).

In Silico Peptide Binding to HLA-DRβ1

Polymorphisms in the HLA-DRB1 beta chain specify the peptide binding preference of HLA-DR. The YST-motif containing serine-11 is located in the bottom of the antigen-binding groove of the HLA-DR protein (**Figure 2**), suggesting that different peptide-binding preferences of HLA-DRB1 may confer risk for uveitis. To explore if the presence of serine-11 affects peptide-MHC Class II interactions, we compared the predicted binding affinity for 13 common *HLA-DRB1* allotypes (representing 79% of *DRB1* alleles in cases) using a panel of >80,000 peptides based on human iris proteome data (**Supplementary Information** and **Supplementary Table 4**) implemented in the *NetMHCIIpan* server (23). The neural network-based *NetMHCIIpan* algorithm is capable of reliably detecting differences between peptide-binding repertoires of highly similar MHC class II molecules (23).

To compare predicted binding preferences, we performed unsupervised hierarchical clustering on the binding profiles of all *DRB1* alleles; clustering discerned two major clusters of classical alleles strikingly similar to the distribution of serine at position 11 (**Supplementary Figure 8**). We observed that the average binding affinity of the peptide panel was higher for the *HLA-*

DRB1 alleles that encode serine at position 11 versus alleles that have other amino acids at this position (Wilcoxon signed-rank test $p = 3.44 \times 10^{-136}$, **Supplementary Table 5**). To compare these two clusters, we computed the ratio of the average binding affinity of the 6 *HLA-DRB1* allotypes that contain serine-11 and the 7 that have other amino acids at this position (**Supplementary Table 5**). Since MHC class II molecules at the cell surface present repertoires that are skewed in favor of high affinity binders (23) we selected for peptides with (IC_{50}) affinities <500 nM (an affinity of <500 nM is routinely used as a threshold for potential immunogenicity) or <50nM (strong binding peptides) for *HLA-DRB1* allotypes with serine-11 (**Supplementary Information**). The data indicated that peptides with an intermediate or high affinity for allotypes containing serine-11 have less affinity to allotypes that contain other amino acids at position 11 (**Supplementary Table 5**).

Discussion

Through interrogation of imputed HLA types and amino acids, we have identified the amino acid serine at position 11 in the *HLA-DRB1* gene as strongly associated to increased risk of uveitis in female JIA patients. The perfect linkage disequilibrium across the YST-motif (positions 10-12) makes it impossible to disentangle the three amino acids in a statistical framework; a subset of the amino acids, all three (i.e., the complete YST-motif), or a more complex interaction of these variants with others in the region may all be key to disease onset. Interrogation of larger sample collections and extensive functional work in this region will be necessary to fully understand the mechanism(s) driving the association. Nevertheless, our findings indicate that, though JIA with uveitis and

JIA without uveitis share clinical features and HLA-specific genetic risk factors (15), there are uveitis-specific genetic signals.

Further analyses revealed that the serine-11 signal is sexually dimorphic and unique to females. The reduced number of male cases (**Table 1**) and resulting reduced power in the male-only analysis may explain the absence of association signal. However, we had 98.5% power to detect an association ($p < 0.05$) at serine-11 in the male-only analysis, assuming the odds ratio in males was the same as in females (OR = 3.30). Assuming a more modest effect in males (OR = 2.00), we still had 75.2% power to detect a signal at $p < 0.05$. To detect more subtle effects in males or other lower-penetrance sexually-dimorphic effects in either sex, larger case collections will be necessary.

Though JIA-uveitis is known to occur more frequently in females (22), more detailed sex-specific epidemiologic data in uveitis-associated JIA is sparse. The genotype-by-sex interaction informs an exciting field of future research aimed at elucidating potential sex-specific uveitis risk mechanisms (e.g., hormone regulation) underlying the *HLA-DRB1* signal. The sexually-dimorphic serine-11 association (or the complete YST-motif) may also help resolve previously-reported evidence for sexual dimorphic severity or disease course in children with JIA-uveitis (24). Lastly, the finding indicates that sex stratification may be beneficial for future clinical trials, as some therapeutic agents may be more efficacious in females or males only (25).

Though all three YST-motif residues reside at the bottom of the HLA-DRB1 peptide-binding groove, only serine-11 is positioned towards, and thus most likely interacting with, binding peptide epitopes (**Figure 2**). Previous work has identified serine-11 as the strongest risk factor in seronegative rheumatoid arthritis (RA) (26). Some (uveitis) JIA patients might be categorized as having seronegative RA by the time they reach adulthood, and seronegative RA is considered to genetically mirror the uveitis-prone JIA categories (14). In contrast, serine-11 is highly protective against seropositive RA, a biologically distinct form of RA in which uveitis is not common (<1% of cases) (27).

HLA-DRβ1 is essential to immunity and orchestrates downstream immune response by presenting a dynamic cargo of thousands of different peptides for scrutiny by T helper cells (28); both JIA and uveitis are thought to be mediated by T helper cell subsets (2). A potential explanation for the strong association of an amino acid motif (tagged by serine-11) in *HLA-DRB1* is that predetermined peptide preferences may affect T helper cell regulation. Although the large panel of putative antigens we tested was by no means exhaustive (**Supplementary Table 4**), the experiment demonstrated that the presence of serine-11 in *HLA-DRB1* is accompanied by changes in binding affinity in the resulting protein (**Supplementary Table 5** and **Supplementary Figure 8**), suggesting that antigen presentation by the HLA-DRβ1 protein may (partially) underpin uveitis susceptibility. *HLA-DRB1* risk alleles that encode serine-11 (**Supplementary Table 2**) may communicate distinct 'peptidomes' that influence T helper cells and increase the likelihood of downstream immune responses (e.g., antinuclear antibodies or ANA) to the eye; ANA-positivity is modestly correlated with

presence of serine-11 in the cases studied here (Pearson's r = 0.21, p = 1.0×10^{-6}). Functional studies using HLA-proteomic approaches will be necessary to experimentally validate and systematically dissect the complex downstream effects of serine-11 on tissue-specific antigen presentation by HLA-DRβ1 in uveitis.

The relatively high frequency of serine-11 in the JIA cases that did not develop uveitis (**Table 2**) indicates the likely involvement of additional (epi)genetic and environmental factors in uveitis. Only a handful of candidate gene studies (2,29), HLA-specific analyses (15) and a recent GWAS (30) have investigated uveitis as a phenotype separate from all JIA, the latter identifying *HLA-DRB1*1501* as a uveitis risk factor. Genes outside the MHC have also been implicated in JIA-uveitis, including a polymorphism in *VTCN1 (31)* and variants near the immune genes *TRAF1* and *C5* (32). Other studies have implicated a role for infiltrated plasma cells (33), T helper cells (2), and changes in the ocular fluid microenvironment (34–36). While we were well powered to detect common (frequency >10%) and highly-penetrant (OR > 3, **Supplementary Figure 9**) genetic variation that associates with increased uveitis risk, we were underpowered to identify common (frequency 1-10%), modestly-penetrant variants (OR 1.05 - 1.5), which are the hallmark genetic feature of complex phenotypes (37,38). Future studies will need to interrogate much larger samples from an array of global populations (39) in order to identify additional genetic signals that influence disease risk.

A number of risk factors, including young age and presence of ANA (assumed to reflect aberrant immune activation; (40)) are used to regularly screen (~4 times/year) JIA patients for early detection of uveitis. However, a considerable number of uveitis cases are diagnosed after sight-threatening complications have already occurred (6). A biomarker test, such as one for serine-11, would significantly simplify diagnosis and prevent unnecessary ophthalmologic screening in JIA patients with low susceptibility for uveitis (4). Deeper examination of our phenotypic data showed that 99% of female uveitis cases carry at least one copy of the variant coding for serine-11. The only female uveitis case who lacked serine-11 in *HLA-DRB1* appeared to suffer from ANA-negative oligoarthritis with mild vitritis and peripheral multifocal choroiditis in the absence of anterior segment inflammation. This ocular finding is atypical for JIA, and thus, according to our inclusion criteria, this sample was likely improperly included at cohort collection. Prospective studies in larger populations, including detailed clinical evaluation of development of uveitis and secondary uveitis phenotypes, will be necessary to dissect the potential of serine-11 or other genotypes as biomarkers for disease risk. Regardless, the current study justifies further genetic analysis.

The results of our study represent a key step in understanding the pathogenesis of uveitis in JIA, helping to discern biologically shared and distinct features of JIA-uveitis and JIA without uveitis. Future work will allow us to further disentangle the two phenotypes, evaluate shared and distinct disease etiology, identify disease pathways, and evaluate the efficacy of serine-11 or other genetic markers as potentially efficient clinical decision-making tools. By

pinpointing and understanding the molecular mechanisms of uveitis, we can identify biomarkers that stratify patients for disease risk, catalyze future lines of research in precision medicine, and advance towards treating and preventing sight-threatening complications of uveitis in children with JIA.

Materials and Methods

Analysis code, supporting data files and links to summary-level data from this work can be found here: https://github.com/saralpulit/UveitisJIA_MHC-fineMapping

Patient collection

JIA and uveitis samples

JIA was diagnosed according to the criteria of the International League of Associations for Rheumatology (ILAR), or by former criteria (e.g., European League Against Rheumatism (EULAR)) (41,42). Ophthalmologists screened all patients according to the Academy of Pediatrics guidelines and patients with no clinical signs of uveitis had ophthalmologic follow-up of at least 4 years after JIA onset (6). Patients with (minimally) trace cells or more in the anterior chamber and treated with at least topical steroids during ophthalmologic examinations were diagnosed with JIA-associated (anterior) uveitis.

DNA material of all Phase 1 JIA patients were collected at the University Medical Center Utrecht, University Medical Center Leiden, Erasmus Medical Center Rotterdam, Academic Medical Center Amsterdam and Radboud University Medical Center Nijmegen (all in the Netherlands). Phase 2 samples were collected from the ICON study and provided by the ICON biobank at the Westfälische Wilhelms-Universität Münster (ICON-JIA Study, Germany), the University Hospitals Leuven (Belgium), the University Children's Hospital at Zurich (Switzerland).

Population-level control samples

Genotype data from 394 unrelated and unaffected Dutch samples were used as population controls and had been previously genotyped using the same platform as the JIA and uveitis samples contained in this study (43).

This study was approved by the local Institutional Review Boards and is in compliance with Helsinki principles. Informed consent was obtained from all participating patients if they were 18 years or older, from both parents and patients if they were 12-18 years of age, and from parents only if they were younger than 12 years old.

Prephasing and imputation

We prephased the Phase 1 and Phase 2 data separately using SHAPEIT2 (44). As the sample size was >100 samples, we ran prephasing without a reference

panel, per the SHAPEIT2 recommendations (44). Following prephasing, we imputed the prephased samples using the IMPUTE2 software (45) and an imputation reference panel constructed using the 2,504 samples whole-genome sequenced by the 1000 Genomes Project Phase 3 (46).

To impute amino acids and HLA alleles in the MHC, we used the SNP2HLA pipeline (17). The panel includes SNPs and amino acids in the MHC, as well as Class I and Class II HLA types. HLA types are imputed to 2- and 4-digit resolution. The SNP2HLA pipeline uses BEAGLE (47) to phase and impute the data. The full details of prephasing and imputation can be found in the **Supplementary Information**.

Genome-wide association testing

GWAS were performed using PLINK 1.9 (48) using an additive logistic regression model, correcting for the top two principal components and sex (**Supplementary Information**). We meta-analyzed data using METAL (16). To ensure we were analyzing SNPs with high-quality imputation, we only analyzed common SNPs (MAF > 1%) with imputation quality (info) score > 0.7.

Association testing in the MHC

We performed additive logistic regression in the Phase 1 and Phase 2 data separately to check the overall behavior of the data. We then merged the dosages together and performed logistic regression on the dosage data using

PLINK 1.9 (48) correcting for the top 5 principal components, sex, and phase. To ensure that this mega-analysis approach was appropriate, we additionally performed an inverse variance-weighted meta-analysis of the two phases, and found that the results were highly concordant (Pearson's r of genome-wide betas = 0.95; **Supplementary Figure 5**). To identify independent signals within the MHC, we performed conditional analysis (**Figure 1**).

In silico peptide binding prediction to *HLA-DRβ1*

We used proteome data from human iris tissues (2,959 nonredundant proteins) as a representative source of proteins present in iris tissue (49). JIA-uveitis patients commonly have ANAs and antibodies directed to iris tissues, so we focused on nuclear iris proteins to generate a potentially disease-relevant dataset. We selected 147 proteins (**Supplementary Table 4**) that fulfilled these criteria and their full length amino acid sequences were fed into the neural network of the *netMHCIIpan3.1* server.

Next, we tested the predicted affinities of all 83,686 overlapping 15-mer peptides from the selected 147 proteins in *netMHCIIpan3.1*. for binding to representative four-digit alleles of *HLA-DRB1*. The affinity data was log-transformed to a value between 0 and 1 using: $1-\log(IC_{50}nM)/\log(50{,}000)$ (23). To categorize *HLA-DRB1* allotypes with similar predicted binding preferences, we performed unsupervised hierarchical clustering and generated heatmaps based on the Euclidean distance measure and the Ward's linkage method using the MetaboAnalyst server (50). We computed the ratio of the average binding

affinity of HLA-DRβ1 molecules that contain Serine-11 in the peptide-binding groove over the average binding affinity of HLA-DRβ1 proteins that have other amino acids at this position as a measure for the overall difference in predicted binding affinity for each peptide.

Figure Legends

Figure 1 | Association and conditional testing in *HLA-DRB1*. A. Initial association testing in the MHC revealed a genome-wide significant signal at *HLA-DRB1* position 11 (presence of serine (S) or aspartic acid (D), purple diamond). **B.** Conditioning on the presence of aspartic acid at position 11 left the association signal essentially unchanged (green and white diamonds, grey outline); presence of serine remained the strongest association (pale purple diamond). Conditioning on the presence of serine at position 11 (aquamarine and white diamonds, black outline), dramatically mitigated the association signal, indicating that presence of serine explains the bulk of the association at *DRB1* position 11. Presence of lysine (K) at position 69 in *HLA-DPB1* remained modestly associated (dark purple diamond). **C.** Conditioning on lysine at position 69 in *HLA-DPB1* removes the remainder of the signal (rs6457109, dark purple diamond, $p = 0.0024$).

Figure 2 | Three-dimensional ribbon model for HLA-DR (Protein Data Bank entry: 3pdo)**.** The molecule is positioned to provide a view from the top of the peptide-binding groove. The beta-chain (DRß) is highlighted in orange. Amino acid serine at position 11 (red) is located in the bottom center of the peptide-

binding groove of HLA-DRß1. Adjacent amino acid tyrosine at position 10 (green) and threonine at position 12 (blue) and serine at position 11 are displayed as spheres. The 3D structure was produced using UCSF Chimera (50).

Tables

Table 1 | Samples included in Phase 1 and Phase 2 of the analysis. Sample numbers are shown both pre- and post-quality control (QC) for JIA cases with (JIA-uveitis) and without uveitis (JIA non-uveitis). The presence or absence of antinuclear antibodies (ANA), sex distribution as well as JIA categories are shown. Poly RFneg, polyarthritis rheumatoid factor negative.

	Total JIA patients	**JIA-uveitis (%)**	**JIA non-uveitis (%)**
Samples, pre-QC			
Phase 1	384	137 (64)	247 (68)
Phase 2	192	77 (36)	115 (32)
Total	576	214	362
Samples, post-QC			
Phase 1	357	126 (66)	231 (70)
Phase 2	165	66 (34)	99 (30)
Total	522	192	330
ANA status			
ANA+	304	150 (78)	154 (47)
ANA-	182	32 (17)	150 (46)
Data	36	10 (5)	26 (8)

unavailable			
Sex			
Female	364	136 (71)	228 (69)
Male	158	56 (29)	102 (31)
JIA category*			
Oligo-persistent	214	101 (53)	113 (34)
Oligo-extended	98	36 (19)	62 (19)
Poly RFneg	169	46 (24)	123 (37)
Other**	41	9 (4)	32 (10)

*An extended description of sex and ANA status within the JIA categories can be found in **Supplementary Table 6**

**Polyarthritis (rheumatoid factor positive, n=4), psoriatic arthritis (n=6), enthesitis-related arthritis (n=15), systemic arthritis (n=3), other arthritis (n=10), unknown (n=3)

Table 2 | Association results for amino acids in *HLA-DRB1*. The top results from the mega-analysis in uveitis and JIA without uveitis in *HLA-DRB1*. All reported results correspond to presence of the given amino acid residue(s). Presence of serine (Ser) or aspartic acid (Asp) at position 11 in *HLA-DRB1* (imputation info score = 1.11) was the top hit after initial association testing. Conditioning on either aspartic acid or serine revealed that the amino acids at positions 10-13, all well-imputed (imputation info = 1.08) and in perfect linkage disequilibrium, explain the bulk of the initial signal.

***DRβ1* position**	**Amino acid residue(s)**	**Classical HLA alleles**	**Samples** (all, females, males)	**Mega-analysis**		
				Freq. Case | Cntl	**OR [95% CI]**	**P-value**
Initial association testing						
11	Ser or Asp	*03,*08,*09, *11,*12,*13,*14	All	0.79 | 0.59	2.59 [1.92 - 3.50]	4.80×10^{-10}
11	Ser	*03,*08,*11, *12,*13,*14	All	0.77 | 0.57	2.47 [1.84 - 3.32]	1.44×10^{-9}
Conditioning on aspartic acid (Asp) at position 11						
11	Ser or Asp	*03,*08,*09, *11,*12,*13,*14	All	0.79 | 0.59	2.60 [1.92 - 3.52]	5.43×10^{-10}
11	Ser	*03,*08,*11, *12,*13,*14		0.77 | 0.57	2.60 [1.92 - 3.52]	5.46×10^{-10}
Conditioning on serine (Ser) at position 11						
11	Ser or Asp	*03,*08,*09, *11,*12,*13,*14	All	0.79 | 0.59	2.36 [0.93 - 5.98]	0.069
Sex-specific association testing						
233	Thr	*01,*04,*07,*08, *09,*10,*15, *16	Females	0.18 | 0.44	0.30 [0.20 - 0.43]	3.50×10^{-10}
			Males	0.37 | 0.46	0.73 [0.44 - 1.20]	0.210
11	Ser or Asp	*03,*08,*09, *11,*12,*13,*14	Females	0.84 | 0.59	3.48 [2.35 - 5.16]	4.92×10^{-10}
			Males	0.67 | 0.56	1.52 [0.92 - 2.51]	0.100
11	Ser	*03,*08,*11, *12,*13,*14	Females	0.82 | 0.57	3.30 [2.26 - 4.83]	7.61×10^{-10}
			Males	0.64 | 0.55	1.41 [0.86 - 2.31]	0.177

Conditioning on threonine (Thr) at position 233						
11	Ser or Asp	*03,*08,*09, *11,*12,*13,*14	Females	0.84 | 0.59	1.98 [0.73 - 5.32]	0.178
			Males	0.67 | 0.56	3.14 [0.70 - 14.15]	0.136
11	Ser	*03,*08,*11, *12,*13,*14	Females	0.82 | 0.57	0.84 [0.10 - 6.94]	0.878
			Males	0.64 | 0.55	2.83 [0.16 - 51.47]	0.482
Sex-interaction association testing						
11	Ser*Sex	*03,*08,*11, *12,*13,*14	All	--	2.25 [1.22 - 4.15]	0.0096

Association statistics for tyrosine (position 10), threonine (position 12) and serine or glycine (position 13) are identical to the association statistics reported for serine (position 11) due to linkage disequilibrium.

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Acknowledgements

The following ICON-JIA study group collaborators have contributed: Universitätsmedizin Charité Berlin, Tilmann Kallinich; Medizinische Hochschule Hannover, Angelika Thon; Universität Tübingen, Jasmin Kümmerle-Deschner; Prof.-Hess-Kinderklinik Bremen, Hans-Iko Huppertz; Asklepios Kinderklinik Sankt Augustin, Gerd Horneff; Olgahospital Stuttgart, Anton Hospach; Kinderkrankenhaus der Stadt Köln, Kirsten Mönkemöller; Deutsches Zentrum für Kinder- und Jugendrheumatologie Garmisch-Partenkirchen, Johannes-Peter Haas; St. Joseph-Stift Sendenhorst, Gerd Ganser; Kinderrheumatologische Praxis am AK Eilbek Hamburg, Ivan Foeldvari; Deutsches Rheuma-Forschungszentrum Berlin, Jens Klotsche.