THE ENIGMA OF UVEITIS IN JUVENILE IDIOPATHIC ARTHRITIS

Genetic, immunologic and clinical aspects

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THE ENIGMA OF UVEITIS IN JUVENILE IDIOPATHIC ARTHRITIS

Genetic, immunologic and clinical aspects

Het raadsel van uveitis bij juveniele idiopathische artritis
Genetische, immunologische en klinische aspecten
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op donderdag 1 maart 2018 des middags te 2.30 uur

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INTRODUCTION

Epidemiology

Uveitis is a sight-threatening inflammation of the inner layers of the eye that affects about 29 in every 100,000 children. Generally, uveitis is responsible for approximately 10% of all cases of blindness in Western countries.[1] The clinically heterogeneous cluster of conditions that fall under the term 'uveitis' are commonly divided into two overarching groups; the 'infectious uveitis' - where the initiating factor is attributed to microbial infection, or 'noninfectious uveitis' - where uveitis is considered to be driven by auto-inflammatory mechanisms. The latter concept is fairly based on the observation that in one third of the children uveitis arises as part of a multi-organ inflammatory syndrome, of which junivele idiopathic arthritis (JIA) is by far the most frequent associated systemic condition.[2] As a whole, JIA is the most common chronic rheumatic disease in childhood with an estimated prevalence of 16-150 in every 100,000 individuals.[3-6] There may be a molecular link between arthritis and uveitis, because also up to one third of JIA patients (depending on JIA subtype) are afflicted by a distinct form of noninfectious uveitis, making uveitis the most common extra-articular manifestation of JIA.[7]

Clinical course

Uveitis associated with JIA (further referred as '*JIA-associated uveitis*') typically manifests as chronic noninfectious uveitis, which predominantly affects the anterior part of the eye. A hallmark of JIA-associated uveitis is the typical asymptomatic onset and insidious disease course that conceal the occurrence of numerous complications ranging from posterior synechiae, band keratopathy, glaucoma, cataract, and cystoid macular edema, to refractory hypotony or even phthisis bulbi.[8-10] These complications usually become noticeable after irreversible or sight-threatening damage has occurred, which significantly hampers success of clinical intervention to minimize visual loss. Consequently, recent studies describe that 6% of children with JIA-associated uveitis become unilaterally blind, whereas in the past percentages of ~30% were reported.[11,12]

Severe disease at onset has a poor visual prognosis, making early detection and prompt treatment in these patients critical.[8,12] Several other risk factors associated with a more severe uveitis course include the male sex, a young age at uveitis onset, development of uveitis shortly after arthritis onset, or detection of uveitis before arthritis (JIA-suspected uveitis).[12-14] In contrast, prolonged periods of relative disease inactivity, and early and extended periods of immunomodulatory therapy substantially reduce the risk of uveitis relapse and vision loss.[8,15,16]

JIA associated uveitis typically has its onset in childhood; however, patients continue to suffer from its complications into adulthood. In fact, persistence of uveitis activity has been reported in 30-63% of adult patients with a history of JIA. Despite the alarming number of adult patients with persisting uveitis activity, only little is known about this condition in adulthood and consequently study outcomes are conflicting, in part due to inconsistent follow-up.[10,17-22]

The w*orld health organization* defines health as 'a state of complete physical, mental, and social wellbeing not merely the absence of disease'. Meaning not only the above mentioned objective disease outcomes such as uveitis activity and extend of complications influence health, but also patients' personal experiences. To determine patients' subjective health experience, a variety of *Quality of Life* (QoL) assessment tools have been developed, with the

vision-related QoL assessment tool for eye diseases specifically (*National Eye Institute Visual Functioning Questionnaire*).[23] In children with JIA-associated uveitis the vision-related QoL was found to be worse in those with uveitis compared to those without uveitis, but both had similar general QoL scores.[24] In adults, uveitis (not JIA-associated) was related to worse QoL outcomes compared to healthy people.[25,26] In adult patients with a history of JIA QoL was also decreased compared to healthy people, however, no uveitis-related outcomes were reported.[27] A study investigating the QoL specifically in adults with JIA-associated uveitis is currently lacking.

Risk factors and screening guideline

The majority of children with JIA-associated uveitis are diagnosed with JIA first (77-97%) and develop uveitis afterwards, yet, to date we are unable to predict which JIA patients are susceptible to developing uveitis.[4,28] However, various risk factors had been identified and patients with pauciarticular and polyarticular JIA* had the highest risk to develop uveitis.[29] Also patients positive for antinuclear antibodies (ANA) and with arthritis onset below the age of 7 years were considered more susceptible to uveitis. According to these risk factors ophthalmologic screening guidelines for JIA patients were first developed in 1993 (and updated in 2006).[29,30] According to these guidelines, high risk patients were screened by ophthalmologists every 3 to 4 months for at least 7 years to capture onset of uveitis and prevent severe eye inflammation. Nevertheless, the proportion of children with severe uveitis at onset did not change, which strongly suggested the need for refinements in the screening protocol.[14]

In the following years, new criteria for JIA were released by the *International League of Associations for Rheumatology* (ILAR).[32] The new guidelines refined that those suffering from the most common (re-defined) categories of JIA - oligoarticular or polyarticular rheumatoid factor negative JIA - were at particular risk for developing chronic anterior uveitis - the hallmark eye inflammation in JIA. In 2007 Heiligenhaus and associates improved the ophthalmologic screening guidelines using these new JIA criteria and by implementing their observations that the highest risk to develop uveitis after JIA onset was within the first year (73% of the patients).[33] In addition, they outlined that the majority of patients developed uveitis within a 4 year window after JIA onset (90%), which is in agreement with previous findings.[30] Although these guidelines significantly aid to single out patients prone to uveitis, still there is a pressing need to further reduce the number of patients that present with severe uveitis at first visit in the ophthalmology clinic and conversely minimize screening in patients with a low risk profile for uveitis.

Molecular markers to guide clinical management of JIA-associated uveitis

Objective and accurate (bio)markers to determine whether a specific JIA patient will (or will not) develop uveitis are currently lacking. Side-by-side comparison of patients with and without uveitis can be performed using biological samples, including serum. As mentioned above, serum levels of ANAs are associated with increased risk for uveitis in JIA.[34] More specifically, anti-histone antibodies (AHA) and antibodies directed to iris/ciliary tissue are more frequently observed in serum of patients with JIA-associated uveitis.[35-37] In addition, various other serum markers have been linked to JIA-associated uveitis, such as increased levels of soluble interleukin(IL)-2 receptor or the heterodimer calprotectin (S100A8/A9), and decreased amyloid A1.[36,38] Despite the association of these blood markers with uveitis status,

** previously known as 'juvenile rheumatic arthritis, JRA' according to the criteria of the American College of Rheumatology (ACR) and as 'juvenile chronic arthritis, JCA' according to the criteria of the European League Against Rheumatism (EULAR), both defined in the 1970s*[29,31]

all lack specificity and thus, do not aid directly in clinical decision making or improve screening for uveitis risk.
Since uveitis in JIA affects the anterior segment, and there is little understanding of the cause of JIA-associated uveitis, there have been attempts to study fluid from this eye compartment to aid into the quest for markers that help to better understand the molecular disturbance of the affected tissues. Aqueous humor (AqH) – a clear watery fluid that fills most of the anterior part of the eye - provides an excellent substance to study mediators. Increased levels of the proteins transthyretin (TTR) and S100A8/A9 were found in the AqH of patients with JIA-associated uveitis.[36,39] Various molecules have been found in AqH of children with JIA, most of which are present throughout many forms of uveitis and include primarily cytokines, chemokines and soluble adhesion molecules.[40] The unique opportunity to study AqH in JIA with uveitis also provides an apparent (ethical) limitation, because the ideal experimental design would demand ocular fluid samples from JIA patients without uveitis. However, ethical consideration pleads that no AqH sampling by fine needle aspiration is performed for mere scientific purposes. Nevertheless, AqH analysis can aid to stratify different clinical forms of uveitis, including *chronic anterior uveitis* without arthritis (CAU) and JIA-associated uveitis.

Treatment
The current medical treatment for uveitis in JIA is mainly based on low evidence studies and experiences in daily practice. Due to the lack of high quality evidence, Dutch ophthalmologists and (pediatric) rheumatologists recently developed guidelines for uniform treatment of uveitis in the Netherlands (‘*Dutch uveitis guidelines*’).[41] In this document they also included guidelines for the management of JIA-associated uveitis. Following this national initiative, international initiatives for guideline development specifically for JIA-associated uveitis were taken; The European initiatives, *Single Hub and Access point for Pediatric Rheumatology in Europe* (SHARE) and *Multinational Interdisciplinary Working Group* for *Uveitis* in *Childhood* (MIWGUC), originated.
The here outlined treatment strategies for JIA-associated uveitis are based on these before mentioned guidelines, including low evidence studies and expert opinions.
Topical corticosteroids remain first-line treatment of JIA-associated uveitis.[42] If inactivity of uveitis with topical corticosteroids cannot be achieved, immunosuppressive drugs (usually low-dose Methotrexate (MTX)) are commonly used as second-line medication. In some severe uveitis cases, systemic corticosteroids are necessary as an additional treatment to suppress uveitis activity. The last decades have seen a dramatic improvement of clinical management of JIA-associated uveitis with the advent of monoclonal biologic therapeutics (specifically the TNF-α inhibitors *Adalimumab* or *Infliximab*). To support the use of biologics in disease management, the recent *SYCAMORE trial* (2017) provides strong evidence that combining Adalimumab with MTX is superior in controlling eye inflammation compared to MTX monotherapy.[43] In cases treatment with monoclonal TNF-α inhibitors are insufficient to control uveitis, alternative biologic agents including *Tocilizumab* (anti-IL-6), *Rituximab* (anti-CD20) and *Abatacept* (T-cell inhibitor) have shown to be effective in small studies.[42,44] Currently, it is unclear to predict treatment response in advance. Unfortunately, despite the above-mentioned therapeutic options, oral or intravenous corticosteroids are nowadays still essential in disease management of serious and treatment-refractory uveitis cases.
A major concern with the current therapeutic armamentarium is the risk for significant side effects, especially at the vulnerable (growing age) stage of life in which JIA-associated uveitis usually manifests. Specifically, corticosteroids, which are often effective in suppressing uveitis

activity, can at the same time paradoxically contribute to visual impairment; Corticosteroids can induce various eye-related problems such as cataract formation or severe glaucoma (**Figure 1**), of which the latter is one of the major causes of global blindness.[45] Furthermore, these side effects demand (sometimes multiple) dreaded surgical interventions to treat this. The risk for cataract formation increases when topical corticosteroids are chronically used for more than three times a day.[46] However, early treatment with MTX is associated with a delay of cataract development.[47] Furthermore, the use of systemic corticosteroids is infamously linked to growth retardation or a cushingoid face.[42] This urges the ophthalmologist to minimize the period of treatment with this class of drugs.

A concern of MTX use is the commonly observed intolerance and accompanied gastrointestinal discomfort.[48] Unfortunately, also the use of biologics for treatment has few major drawbacks; First, their wide immunosuppressive function exposes patients to increased risk for opportunistic infection, which in rare cases may even include severe conditions such as tuberculosis. Although the 'humanization' of recombinant monoclonal antibodies has drastically reduced their immunogenicity, even the available state-of-the-art biologicals are well known to over time lead to the formation of neutralizing anti-biological antibodies in treated patients, which permanently reduces the therapeutic efficacy and discourages monotherapy by biologicals in JIA-associated uveitis. Consequently, treatment with the widely used anti-TNF (e.g. adalimumab) is usually combined with MTX to reduce anti-drug antibody formation.[49]

Together, these observations illustrate the pressing need for clinical or molecular markers, that aid in better understanding the large differences in treatment responses and safety issues in disease-modulating drugs. Subsequently, an objective framework, that eventually will guide a tailor-made clinical management program for individual patients (e.g. personalized medicine in JIA-associated uveitis) with preservation of vision and hopefully improvement of QoL, is required.

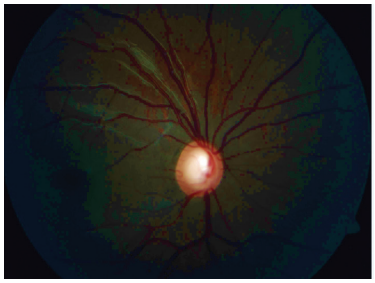

Figure 1. *This figure shows an excavated optic disc of a 5-year old patient with juvenile idiopathic arthritis associated uveitis. This was the result of glaucoma accompanied by the use of topical steroids.*

Pathogenesis

The underlying molecular mechanisms that link and drive JIA and uveitis have puzzled the scientific community for halve a century. Both conditions are considered to be multifactorial immune-mediated diseases with a complex genetic predisposition and are likely influenced by distinct environmental factors, such as pathogenic agents, or chronic concealed infection, but also additional factors such as psychological stress, vitamin D deficiency and vaccinations were suggested to trigger JIA as well as uveitis development.[50] Of all cellular subsets of the immune system, the CD4+ T-lymphocytes are the most well studied in these conditions and

are deemed to play an important role in the pathogenesis of both autoimmune uveitis as well as in oligo- and polyarticular JIA. A specific breed of CD4+ T cells, the Thelper-1 (Th1) and Thelper-17 (Th17) cells – notorious for their role in inflammatory diseases - are well-studied perpetrators of intra-ocular inflammation and arthritis.[51-53]
Indeed, immunohistochemical examination of iris biopsies of patients with JIA-associated uveitis revealed infiltration of CD4+ T cells.[50] Also in oligo- and polyarticular JIA an aberrant increase in Th1/Th17 cells are thought to drive joint inflammation. Similar to iris tissues, synovium of JIA patients also displays increased frequency of these CD4+ T-cell populations.[53]
Besides the conventional focus on T-cells in studies of these pathologies, emerging evidence suggests a pivotal role for B-cells in non-infectious uveitis, particularly in JIA-associated uveitis.[54] For example, a typical risk factor for JIA-associated uveitis is the presence of ANA's, which are auto-antibodies produced by B-cells that are directed to nuclear proteins. B-cells recognize antigens via their B-cell receptor. Mature naïve B cells travel from the spleen via the circulation to secondary lymphoid organs and enter lymphoid follicles. Here, they interact with Thelper-cells to differentiate into plasma cells that produce (auto-)antibodies.[54] In a recent immunohistochemistry study of iridectomy samples of JIA-associated uveitis cases, plasma cells were consequently observed.[50,53] In another study, plasma cells and B-cells were found in an enucleated (due to hypotony and pain) eye of a patient with JIA-associated uveitis.[55] In line with this is the reported beneficial effect of Rituximab, a monoclonal antibody directed to CD20, for treatment of JIA-associated uveitis.[56] Curiously, intraocular IgG production against parvovirus B19 was specifically found in children with JIA-associated uveitis, and thereby suggesting that it possibly plays an important role in its pathogenesis.[57]

Genetics of JIA-associated uveitis
The presence of a strong genetic predisposition in JIA is suggested by the relative high concordance rate (~25-40%) in monozygotic twins.[53,58] Although scarce, reports on familial cases of JIA-associated uveitis support the hypothesis of a genetic susceptibility for this eye condition in JIA patients.[59] Here, like most chronic inflammatory conditions, the genetic architecture underpinning uveitis is considered to be complex and dependent on the presence of various environmental factors.[4] JIA, uveitis and JIA-associated uveitis all share genetic associations with the *major histocompatibility complex* (*MHC*) region on chromosome 6, which encodes various *human leukocyte antigens* (*HLA*).[60] HLA-molecules play a major role in adaptive immunity by presenting peptide motifs to effector cells (e.g. T cells, natural killer cells) to orchestrate an immune response to pathogens or induce tolerance to essential microbes and self-tissues. Given their central role in immunity, it is not surprising that numerous HLA-associations are reported in many autoimmune diseases (e.g. *multiple sclerosis, rheumatoid arthritis, type 1 diabetes mellitus*).[61] The HLA genes are highly polymorphic and resulted in >17,000 reported HLA alleles worldwide according to the *Immuno Polymorphism Database* (IPD)-MHC (Release 2.2.0.0 build 464).[62] Classically, HLA genes (and their alleles) are divided in two overarching functional classes - HLA class-I and class-II - based upon the distinct molecular pathways these groups of alleles usually route, and the two dominant lymphocyte (CD8 and CD4 T cells) populations they regulate.
Within the HLA class-I cluster are the *HLA-A*, *HLA-B*, and *HLA–C* genes. Each individual in the population carries two variants (termed alleles) of each of these class I genes. The HLA class I molecules are present on nearly all cells of the body and predominantly present peptides derived from intracellular proteins. The HLA class-II cluster of genes consists predominantly

out of three pairs of α- and β-chain genes that encode proteins which form heterodimers; *HLA-DRA and -DRB*, *HLA-DPA* and *HLA-DPB,* and *HLA–DQA* and *-DQB*. Unlike HLA class I, expression of HLA class-II proteins are mostly limited to ('professional') antigen presenting cells (such as dendritic cells, monocytes and B-lymphocytes). Class-II molecules are usually loaded with peptides that are taken up by the cells from the extracellular environment. Common polymorphisms in the HLA genes can alter the HLA protein structure (alleles) and often change the binding potential of peptides to the HLA protein – thus, distinct HLA alleles overall bind different peptides and communicate distinct messages to the immune system.[63] Here, peptide presentation by HLA class I is recognized by CD8+ T cells, while peptides presented by HLA class II can activate CD4+ T cells. Why some HLA alleles are strongly associated with specific forms of chronic inflammatory diseases is not fully understood, but it is tempting to speculate that unique peptide binding preferences of the risk HLA alleles lead to activation of pathogenic T cells and tissue damage. For example, in HLA class II transgenic mice, some *HLA-DR* molecules are protective while other *HLA-DR* alleles can present peptides derived from intraocular (self-)proteins such as retinal S-antigen or retinol-binding protein 3 (RBP3) and cause experimental autoimmune uveitis.[4,64,65]

HLA genotyping in JIA-associated uveitis also revealed various HLA class I and class II associations.[24] More specifically, various alleles of the *HLA-DRB1* gene (*HLA-DRB1*13, *11, *08*) and the class I allele *HLA-A*02:06*.[24,66-69] Most of these HLA association studies are performed in relatively small cohort studies and lack robust replication studies. Interestingly, recent large-scale genome-wide association studies (GWAS) and MHC fine mapping studies in patients with oligoarticular and rheumatoid factor negative polyarticular JIA – the uveitis-prone categories of JIA - mapped the primary genetic association to the *HLA-DRB1* gene, more specifically the amino acid position 13 in this highly polymorphic class II gene.[70 71] Unfortunately, stratification for uveitis status was not performed in either of these studies, but given the previously reported associations with the *HLA-DRB1* region in patients with uveitis, similar GWAS or MHC fine mapping approaches would provide attractive tools to interrogate genetic susceptibility for uveitis in JIA. Curiously, a recent GWAS of non-infectious (non-JIA) uveitis also mapped the primary association to *HLA-DRB1*.[72]

GWAS approaches to JIA-associated uveitis

In contrast to Mendelian traits where rare mutations in a single or few genes cause disease, multifactorial diseases such as JIA-associated uveitis are considered to be mediated by various epigenetic, environmental and genetic factors.[73] Here, the largest part of the genetic susceptibility is considered to be mediated by predominantly common variants that each have a relatively mild risk effect, which can be captured by *single nucleotide polymorphism* (SNP) genotyping. In the last decade, the most popular approach to interrogating SNPs across the genome has been the *genome-wide association study* (GWAS - see also previous paragraph).[74] In principle, GWAS compares the frequencies of common genetic variants, under the assumption that a strong deviation in the frequency of a single SNP (or a region of SNPs with strong linkage disequilibrium) implicates that genetic loci in their proximity (i.e. the gene in which the SNP is located) mediate susceptibility to the studied trait (disease).

As previously mentioned, despite the great potential for GWAS to dissect the genetic susceptibility of JIA-associated uveitis, such investigations are yet to be performed. A major challenge in stratification for uveitis status is the need for detailed ophthalmological data and follow-up of at least 4 years to confidently (>90%) ascertain or dismiss the presence of uveitis in study patients. To accomplish the careful integration of clinical data and DNA sample

collection, a good collaboration between pediatric rheumatologists and ophthalmologists is vital. In the Netherlands and other European countries, years of investment by pediatric rheumatologists and ophthalmologists have led to a tight multidisciplinary collaboration and the availability of registries of JIA patients with detailed ophthalmological data (e.g uveitis status). These efforts facilitate the molecular investigation of carefully phenotyped patients with JIA and uveitis. The *University Medical Center Utrecht* (UMC Utrecht) is a nationally certified *Center of Excellence* for both uveitis and JIA and has access to national biobanks for these patient populations. The close collaboration with key laboratories and academic centers on JIA-associated uveitis will enable genetic investigations into the susceptibility of uveitis in JIA patients.

Box 1 – GWA studies

In 2003 the *International Human Genome Project* mapped the entire 3 billion base pairs across the human genome, which provided the opportunity for detailed investigation of genetic variations in (diseased) populations.[75] *Single nucleotide polymorphisms* (SNPs) are common genetic variations in the genome. In a GWAS thousands to millions of SNPs are compared between affected (disease) and unaffected (controls). Differences in the frequency of SNPs (the p-value) between these groups are summarized in a *Manhattan-plot* (**Figure 2**) and are considered associated (protective or risk) with a disease if they exceed a certain threshold (the golden-standard is a p-value $<5x10^{-8}$).

Due to linkage disequilibrium (LD) and the possibility of SNP imputation it is not necessary to physically genotype the entire genome. Two SNPs are in Linkage Disequilibrium (LD) if they are associated with each other in the population more often than one would expect by chance; they are usually located near each other.[76,77] Sequence data from the *HapMap-project* and *1000 Genomes-project* provides LD data of populations, which can be used to predict (impute) SNPs that have not been genotyped to economically expand the dataset for association testing.[74] Using combinations of SNPs, HLA alleles and their encoding amino acids can be imputed for detailed study of the MHC region.[78,79]

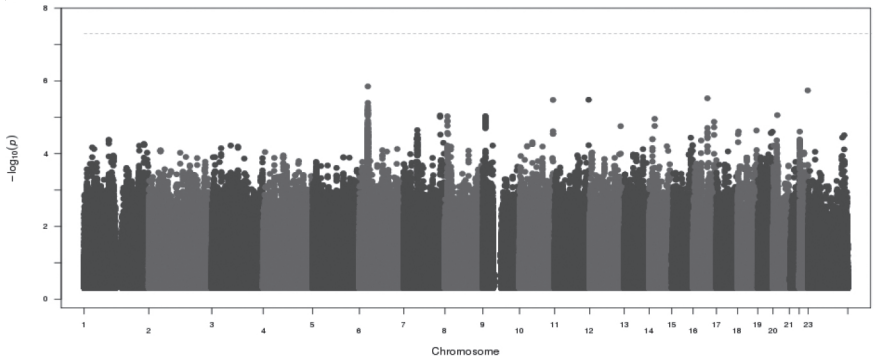

Figure 2. *A Manhattan-plot visualizes the results of a GWAS. On the Y-axis the p-values (for a SNP, cases versus controls) are shown and on the x-axis every single SNP is plotted. The grey dotted line is the line for statistical significance ($p=5x10^{-8}$). In this figure, none of the SNPs reaches statistical significance.*

AIMS OF THE STUDY

Despite the fact that the association of uveitis in JIA was already described by Ohm in 1910 and has a life-long impact, it remains a poorly understood condition; accurate biomarkers to predict uveitis in JIA and specific treatments for JIA-associated uveitis are very limited. Thus there is an unmet clinical need to discover accurate biomarkers, unravel the pathogenesis in order to find, and finally develop, the best medical treatment (preferably for each individual patient).
Furthermore, JIA-associated uveitis is classically seen as a disease of childhood, but there are increasing suggestions that patients deal with complications, treatment and disease activity far into young adulthood. Understanding of the prognosis of JIA-associated uveitis in adulthood is currently lacking.

The overarching aim of this thesis is to identify clinical and molecular biomarkers and risk factors associated with the occurrence of uveitis in JIA patients. Additionally, we studied the prognosis and QoL of JIA patients in early adulthood.
To accomplish this, we separated our strategy into two major and one minor objective. In the *first objective*, we study clinical, molecular and genetic factors associated with the occurrence of uveitis in JIA, which may be used as a framework for predictive clinical-decision tools in the near future. In parallel, *secondary* to this objective, we use the insights from the genetic and molecular investigations to better understand the cause and biology of this debilitating disease. *Additionally*, we study the prognosis and QoL of this typical childhood disease in adulthood.

Clinical, molecular and genetic factors

- Despite the ophthalmologic screening guidelines, detection of uveitis is still delayed in some patients or children develop uveitis within periodic screening moments and present with irreversible damage during the next visit. In contrast, some patients with JIA are screened regularly, but will never develop uveitis at all. The purpose of our study was to investigate clinical and molecular factors at JIA-onset, to improve prediction of occurrence of uveitis in JIA patients.
- To reveal the possible genetic basis, we conducted a GWAS to identify risk loci associated with uveitis in JIA. These risk loci may guide subsequent targeted investigations or may be used to establish a risk profile for the occurrence of uveitis in JIA and can lead to a better understanding of the biology of the disease.
- To better understand the microenvironment of the affected tissues in the anterior segment of the eye in JIA-associated uveitis, we used a multiplex proteomic approach to unravel the composition of immune mediators at the side of inflammation. The multiplex bead-based immunoassay is an established technique to simultaneously determine many soluble immune mediators in small volumes, such as AqH. We analyzed immune mediators in AqH of children with different forms of uveitis and children without uveitis, to identify potential new biomarkers and underlying immunologic patterns of uveitis in JIA.

Prognosis of JIA-associated uveitis in adulthood

- JIA-associated uveitis starts in childhood; nevertheless, patients are still dealing with complications and intensive treatment for this disease in adulthood. Previous studies

showed a lower QoL of patients with either uveitis or JIA. We studied the impact of uveitis on the QoL of adults suffering from JIA by comparing patients with JIA with and without uveitis.

- Also, little is known about clinical outcomes and course of JIA-associated uveitis in adulthood, clinical studies seldom have a follow-up of more than seven years. Therefore we studied uveitis activity, complications and visual prognosis in adults with JIA-associated uveitis.

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Chapter 2

Erythrocyte sedimentation rate as baseline predictor for the development of uveitis in children with juvenile idiopathic arthritis

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ABSTRACT

Purpose
To analyze inflammatory parameters as possible predictors for the development of uveitis in juvenile idiopathic arthritis (JIA) patients. Further, to analyze the predictive value of demographic and clinical factors at the onset of arthritis.

Design
Retrospective cohort study.

Methods
In 358 children with oligoarthritis and rheumatoid factor-negative polyarthritis, erythrocyte sedimentation rate (ESR), C-reactive protein, leukocyte count, presence of antinuclear antibodies (ANA), presence of human leukocyte antigen (HLA-)B27, age of onset of JIA, and sex were analyzed for their predictive value for the onset of uveitis.

Results
One hundred forty-seven patients (41%) were diagnosed with chronic anterior uveitis. Young age of onset, presence of ANA, and elevated ESR appeared to be predictive factors according to univariate analyses (P = .029, P = .007 and P = $5E^{-4}$, respectively). According to multivariate analysis, young age of onset and elevated ESR appeared to be predictive after adjusting for the other relevant factors (P = .004 and P = .001, respectively). A prediction model was developed.

Conclusions
Elevated ESR appears to be a predictor for the occurrence of uveitis in patients with JIA. Since ESR is already routinely tested in patients with recently diagnosed arthritis, its use as a biomarker can easily be implemented in daily practice.

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in childhood and is defined as arthritis without a known etiology which begins prior to the age of 16 and persists for at least 6 weeks.[1,2] Uveitis, typically chronic anterior uveitis, is the most common extra-articular manifestation in patients with JIA and JIA is the most common systemic association of uveitis in children.[3,4] The JIA subtype with the highest association with uveitis is oligoarthritis, followed by rheumatoid factor (RF)-negative polyarthritis, with an incidence of 13%-45% and 10% respectively.[4,5]

The severity of uveitis at presentation is known to predict a severe course and worse visual outcome.[6] Since the course of chronic anterior uveitis is asymptomatic, routine ophthalmologic examination is required in patients with JIA and early treatment is critical to prevent visual loss.[6] Despite early detection, aggressive autoimmune disease can cause harmful uveitis with a worse outcome as well. Complications associated with poorly controlled or untreated uveitis include posterior synechiae, cataract, glaucoma, cystoid macular edema, and band keratopathy.[6-8]

Among patients with JIA the oligoarticular subtype, antinuclear antibodies (ANA) positivity, young age of onset, and female sex in early onset arthritis are predictive factors for the development of uveitis.[4,9-12] Identification of more predictors can help to improve screening protocols for routine examination in order to focus even more on those with the highest risk and to protect them from visual loss.

The aim of this study is to analyze inflammatory parameters and demographic and clinical factors at the onset of arthritis as possible predictors for the development of uveitis among patients with JIA.

PATIENTS AND METHODS

A retrospective cohort study of 358 patients with JIA visiting the ophthalmologist or the pediatric rheumatologist at the University Medical Center of Utrecht, Leiden and Groningen in the Netherlands was performed. Only patients with the JIA subtypes oligoarthritis and RF-negative polyarthritis were included. The JIA diagnosis based on the criteria of the International League of Associations for Rheumatology was confirmed by a pediatric rheumatologist and all patients with JIA were screened by an ophthalmologist at least as many times as recommended by the guidelines of the American Academy of Pediatrics.[2,13,14] Patients with onset of uveitis before arthritis were excluded. Also, patients with uveitis entities other than chronic anterior uveitis were excluded. Patients were divided into 2 groups: JIA patients with (Group 1) and without (Group 2) uveitis. The patients in the second group had an ophthalmologic follow-up of at least 4 years without signs of uveitis. The collection of data from patients' medical charts for the research goals as described in this article was approved by the Institutional Review Board of the Utrecht University Medical Center and is in compliance with the Helsinki principles.

The values of the inflammatory parameters erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and leukocyte count at the time of diagnosis of JIA were collected from patients' medical charts. Only results of patients with inflammatory parameters tested 12 months or less prior to the diagnosis of JIA, 12 months or less after diagnosis of JIA, and before the start of systemic immunosuppressive therapy were included in the analyses.

Information about the presence of ANA at the time of diagnosis of JIA was collected. Additional patient characteristics including date of birth, sex, date of onset of JIA, date of onset of uveitis, and presence of human leukocyte antigen (HLA-)B27 were collected from the medical charts. The age of onset of JIA was calculated from the date of birth and date of onset of JIA. The date of onset of JIA and the date of onset of uveitis were used to calculate the time-interval between JIA and uveitis.

In some medical charts it was unclear whether the patient had ever been diagnosed with uveitis. Therefore, a questionnaire was sent to 42 patients with JIA, who were screened by an ophthalmologist outside the University Medical Centers. Three patients confirmed the uveitis diagnosis, these patients or their parents were asked to sign an informed consent in order to make a copy of the ophthalmologist's patient's medical chart available. This chart was additionally checked for the presence of uveitis, to be sure all patients would be placed in the correct group (uveitis vs non-uveitis). The collected additional information of all these 42 patients was obtained from the medical charts of the University Medical Centers.

Statistical analyses were performed with IBM SPSS Statistics version 20 for Windows and the R rms package. Univariate analyses were performed to find independent predictors that might enter the multivariate analysis. The Pearson X^2 test or Fisher exact test was applied for univariate analysis of categorical variables. Since there were no normally distributed variables, confirmed by the Kolmogorov-Smirnov-test, the Mann-Whitney *U* test was used for continuous, abnormally distributed variables. Subgroup analyses were performed for patients with RF-negative polyarthritis and oligoarthritis. Statistically significant variables in univariate analysis as well as previously known predictors were selected for multivariate analysis by logistic regression. The continuous variable age of onset of JIA was dichotomized according to clinical standards based on prior literature with an age of 6 years as a cutoff point.[14] *P* values ≤.05 were regarded as statistically significant. For presentation, medians were used for the abnormally distributed variables. Based on the results of the multivariate analysis and the current screening guidelines for uveitis in patients with JIA (which includes ANA and age of onset of JIA), a prediction model was developed.[4,14] To test the ability to discriminate between patients with and without uveitis, the area under the receiver operating characteristic curve was determined. When prediction models are derived from multivariate regression analyses, overestimation of regression coefficients is a known phenomenon which results in too extreme predictions in new patients. Therefore, internal validation with bootstrapping techniques was applied, which resulted in a shrinkage factor for the regression coefficients.[15] Also, the value for the area under the receiver operating characteristic curve was corrected for optimism using the bootstrap procedure.

RESULTS

General characteristics of study population

From a total of 358 patients with oligoarthritis and RF-negative polyarthritis entering the study, 147 (41%) were diagnosed with chronic anterior uveitis. All of these patients had at least 1+ cells in the anterior chamber at consecutive visits that needed treatment with topical steroids or immunomodulating medication.[16] The median time between JIA onset and uveitis onset was 1.0 year (range 0.0-24.3 years). Fifty percent of the patients developed uveitis within the first year after onset of JIA, 66% did so within the first 2 years and in 85% uveitis occurred within the first 4 years. In the majority of the patients the inflammatory parameters

were tested at the moment of JIA diagnosis (median time between diagnosis of JIA and laboratory records: 0 months, interquartile range: 0-0 months). The proportion of subtypes of JIA in the group of patients with uveitis could be compared to that in the group of patients without uveitis, with oligoarthritis being the most common subtype. Likewise the female-to-male ratio was similar in both groups (**Table 1**).

Table 1. Demographic and Clinical Data of Patients With Juvenile Idiopathic Arthritis Grouped According to the Occurrence of Uveitis

	Uveitis	Non-Uveitis	*P* Value
Sex, no. (%)			
No. patients	147	211	.394[a]
Female	112 (76)	151 (72)	
Male	35 (24)	60 (28)	
JIA subtype, no. (%)			
No. patients	147	211	.282[b]
Oligoarthritis	108 (74)	141 (67)	
Polyarthritis	39 (26)	70 (33)	
Age (y) of onset JIA			
No. patients	148	213	
Median (range)	2.7 (0.9-9.5)	3.1 (0.7-15.0)	.029[c,d]
ANA, no. (%)			
No. patients	146	211	
Positive	113 (77)	135 (64)	.007[a,d]
Negative	33 (23)	76 (36)	
HLA-B27, no. (%)			
No. patients	54	73	
Positive	9 (17)	11 (15)	.811[a]
Negative	45 (83)	62 (85)	
ESR (mm)			
No. patients	112	197	
Median (range)	32 (2-150)	23 (2-115)	5E^{-4}[,d]
CRP (mg/L)			
No. patients	105	188	
Median (range)	11.0 (0-136)	6.0 (0-303)	.053[c]
Leukocyte count x 10^9/L			
No. patients	108	195	
Median (range)	9.4 (4.5-109)	9.32 (3.8-18.9)	.749[c]

ANA = antinuclear antibodies; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; HLA-B27 = human leukocyte antigen B27; JIA = juvenile idiopathic arthritis.
[a]Fisher exact test.
[b]Pearson X^2.
[c]Mann-Whitney *U* test.
[d]Significant *P* values.

Age of onset

The median age of onset of JIA appeared to be statistically different between the 2 groups: The median age was 2.7 years in patients with uveitis and 3.1 years in patients without uveitis (*P* = .029).

Antinuclear antibodies and human leukocyte antigen B27

Patients with uveitis were significantly more often ANA-positive compared to patients without uveitis (*P* = .007). Presence of HLA-B27 did not entail a significant difference between the 2 groups.

Inflammatory parameters

Statistically, ESR at the time of diagnosis of JIA was significantly more elevated in the uveitis group (median 32 mm) compared to the non-uveitis group (median 23 mm; $P = 5E^{-4}$; **Table 1**). Also, in the oligoarticular and polyarticular subgroups, ESR was significantly more elevated in patients with uveitis. In patients with oligoarthritis, the median value was 30 mm in patients with uveitis and 20 mm in patients without uveitis ($P = .008$). In patients with polyarthritis, the median value was 40 mm in patients with uveitis and 27 mm in patients without uveitis ($P = .010$). After analyzing ESR exclusively in patients who developed uveitis in the first year after onset of JIA, there appeared to be a strong statistical difference between the ESR in the 2 groups with 39 mm in the uveitis group and 23 mm in the non-uveitis group ($P = 1.7E^{-5}$, uveitis n = 55, non-uveitis n = 197). In the second (n = 18, ESR = 30 mm), third (n = 10, ESR = 26 mm) and fourth (n = 11, ESR = 19 mm) year after JIA diagnosis, ESR was slightly elevated in the second and third year in the uveitis group. In these three groups there was no statistical significant difference compared to the non-uveitis (n = 197, ESR = 23 mm) group ($P = .318$, $P = .839$, and $P = .493$, respectively). The inflammatory parameters CRP and leukocyte count at the time of diagnosis of JIA did not statistically differ between the 2 groups (**Table 1**).

Multivariate analysis and prediction model

Because of the statistically significant outcomes in univariate analysis, ANA, age of onset of JIA, and ESR were selected for multivariate analysis. Additionally, the factors sex and JIA subtype were selected. Adjusted for age of onset of JIA, ANA, sex, and JIA subtype, ESR appeared to be a statistically significant predictor for the occurrence of uveitis in patients with JIA with an odds ratio (OR) of 1.016 (95% confidence interval [CI] 1.006-1.026, $P = .001$), which means that for each elevation of 1 mm ESR, the odds for the occurrence of uveitis increases with 0.016 (**Table 2**). Onset of JIA before the age of 7 years was, adjusted for ANA, ESR, sex, and JIA subtype, a statistically significant predictive factor for the occurrence of uveitis in patients with JIA with an OR of 3.167 (95% CI 1.432-7.006, $P = .004$; **Table 2**). The predictors ANA, age of onset, and ESR were included in the prediction model (**Figure 1**). The area under the receiver operating characteristic curve of the model was 0.644 (95% CI: 0.582-0.706; **Figure 2**).

Table 2. Risk for the Occurrence of Uveitis in Children With Juvenile Idiopathic Arthritis According to Multivariate Analysis by Logistic Regression (Uveitis n = 112; Non-uveitis n = 197)

	OR[a] (95% CI[b])	*P* Value
Female sex	0.995 (0.561-1.764)	.986
Oligoarticular subtype	1.406 (0.817-2.419)	.219
Age of onset JIA ≤ 6 years	3.167 (1.432-7.006)	.004[d]
ANA positivity	1.397 (0.813-2.402)	.226
ESR	1.016[c] (1.006-1.026)	.001[d]

ANA = antinuclear antibodies; CI = confidence interval; ESR = erythrocyte sedimentation rate; JIA = juvenile idiopathic arthritis; OR = odds ratio.
[a]Odds ratio for dichotomous variables: the greater the OR, the higher the risk for the occurrence of uveitis.
[b]Confidence interval is the interval estimate of the odds ratio.
[c]Odds ratio for ESR: for every elevation of 1 ESR-point, the odds for the occurrence of uveitis increase by 0.016.
[d]Significant *P* values.

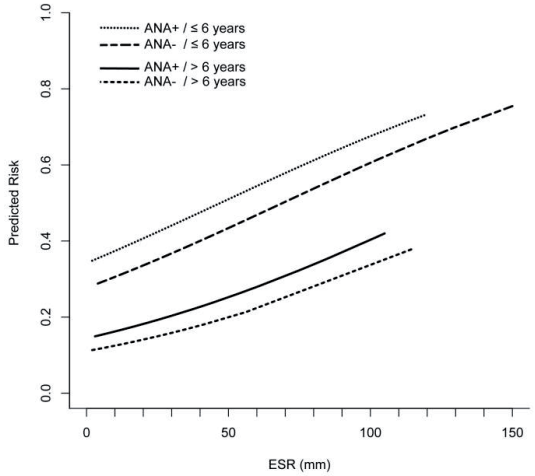

Figure 1. *Prediction model to predict the risk for the occurrence of uveitis in patients with juvenile idiopathic arthritis. ANA = antinuclear antibodies; ESR = erythrocyte sedimentation rate; ≤ 6 years = age of onset of juvenile idiopathic arthritis (JIA) is 6 or below the age of 6; > 6 years = age of onset of JIA is above 6 years. The risk for the occurrence of uveitis based on ANA, age of onset of JIA, and ESR at time of diagnosis of JIA is calculated with the formula = $e^x/(1 + e^x)$, with x = -2.171 + (0.305 x ANA) + (1.126 x age of onset) + (0.014 x ESR). The regression coefficients in this formula are derived from multivariate logistic regression with addition of the bootstrapping technique. Example of a case: A patient presents with JIA. He/she is 3 years old, has a negative ANA in the laboratory and an ESR of 50 mm. This patient has a predicted risk to develop uveitis of 0.41.*

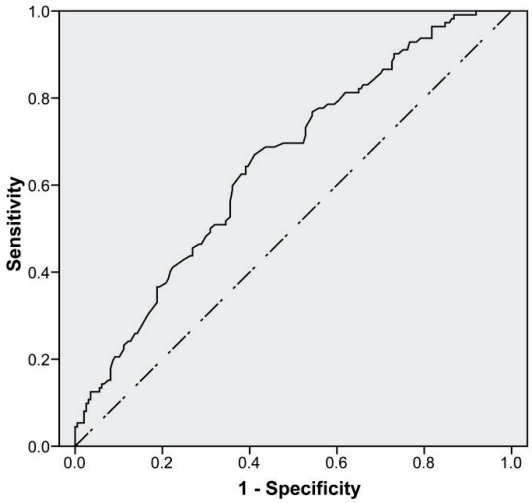

Figure *2.* *The area under the curve shows the ability of the prediction model to predict the development of uveitis in patients with juvenile idiopathic arthritis. The closer the area under the (receiver operating characteristic) curve (AUC) comes to 1, the more accurate is the prediction model. The diagonal line with interruption (AUC = 0.5) represents the situation in which the model would be useless. The continuous line represents the AUC for this prediction model (**Figure 1**), which is 0.644 (95% confidence interval: 0.582-0.706) after correction for optimism using the bootstrap procedure.*

DISCUSSION

For the improvement of our knowledge of the occurrence of uveitis in JIA patients the identification of predictors remains important. In this study we examined the predictive value of the inflammatory parameters ESR, CRP, and leukocyte count. We found an elevated ESR at the time of diagnosis of JIA to be predictive for the occurrence of uveitis in patients with JIA according to univariate analysis. Four previous studies examined the relationship between ESR and the occurrence of uveitis in patients with JIA.[4,10,17,18] Elevated ESR appeared to be predictive in 3 of these previous retrospective studies as well. Statistically, these studies found significantly more elevated ESR values of ≥35 mm in patients with uveitis compared to ESR values of ≤35 mm in patients without uveitis. Our outcomes are perfectly in concordance with these previous studies. Additionally we found ESR to be a predictor in multivariate analysis. This is the second study demonstrating an elevated ESR to be predictive after adjusting for other predictors in a larger study population.[18] One study described ESR as not having a significant influence on the occurrence of uveitis according to multivariate analysis.[4] Patients diagnosed with uveitis prior to the onset of JIA were not excluded from this study, which might explain this contrasting outcome.

In the groups which developed uveitis 2 years and 3 years after JIA onset, ESR was more elevated in uveitis as compared to non-uveitis patients, but the difference was not statistically significant. The lack of a statistical difference can be explained by the lack of power because only 18 and 10 uveitis patients, respectively, were included in these specific groups. In the group developing uveitis 4 years after JIA onset, ESR values were comparable for both groups. ESR in the course of JIA is dependent on many factors including auto-immune disease activity and treatment with immunomodulating medication. In some of our patients with normal ESR at JIA-onset, uveitis debuted 4 years later and several months after methotrexate was stopped. So uveitis might have been suppressed by immunosuppressive therapy and in a few other cases, late uveitis onset was related to JIA flare-up.

The exact mechanism of JIA uveitis and the immunopathogenic link between JIA and uveitis is still unknown, but it is considered to be a multifactorial autoimmune disease.[19, 20] In general, elevated ESR values indicate more activity of the autoimmune disease, so elevated ESR in uveitis patients might reflect a more activated state of the immune system. This might enhance influx of inflammatory cells in the eye with probably a dysbalance of T-helper cells and T-regulatory cells resulting in uveitis. However, more research is warranted to clarify the pathogenesis of uveitis in JIA and the relation with JIA activity.

According to our knowledge, the predictive value of CRP has been tested twice before. In 1 study CRP was dichotomized with a cutoff value of 5 mg/L, based on their laboratory standards, a CRP of > 5 mg/L did not appear to be predictive for the occurrence of uveitis.[10] The other study did not find a difference between the mean CRPs.[18] We confirmed these outcomes and did not find CRP to be predictive in our series. However, we cannot rule out that it might prove to be significant in a larger study population.

Since uveitis is a common comorbidity in patients with JIA, many studies focusing on possible predictors for the occurrence of uveitis have been performed in the last decades.[21] The identification of some predictors led to the development of a screening protocol for ophthalmologic examination in patients with JIA in 1993, which was updated in 2006.[14,22] ANA positivity, oligoarticular JIA, and onset of JIA before the age of 7 appeared to be good predictors and were included in the screening protocols.[9,12,14,23] In this study we have shown that elevated ESR seems to be a good predictor as well. Therefore we recommend ophthalmologists and pediatric rheumatologists to be aware of the increased risk of developing uveitis in children with the aforementioned risk factors and an additional elevated ESR at onset of JIA. ESR is already routinely tested in patients with JIA, so it can easily be implemented in daily practice. We developed a prediction model for development of uveitis which includes ANA, age of onset of JIA, and ESR.[4,14] We validated the model internally with bootstrapping techniques, but external validation is required before the model can really be applied. The proposition of being more aware of high-risk patients in order to protect them from severe complications and visual loss is in concordance with the ideas of Chia et al.[4,24]

In the current study we confirmed the predictive value of ANA positivity and early age of onset of JIA in univariate analyses. We could not confirm JIA subtype as a possible predictor because we only included the subtypes of JIA which are already proven to be related to the occurrence of uveitis.

In concordance with previous studies, we did not find a predictive value of HLA-B27 in our population.[12,23]

As all other retrospective studies, this study has several limitations. The study was based on a patient directory from tertiary centers. So concerning uveitis there might be a referral bias because there is a possibility that more severe cases are included in the study. This was prevented as much as possible by requesting information on uveitis status via questionnaires to patients and parents who were screened by ophthalmologists in secondary referral centers, but it might still cause some referral bias. The case-control character of this study barely makes it possible to represent the actual incidence of uveitis in patients with JIA, given that the ophthalmologic center of the University Medical Center in Utrecht is a specialized uveitis center with a high incidence of uveitis patients. We chose a minimum follow-up with ophthalmologic examinations of 4 years, but we know that in rare cases uveitis will develop even up to 20 years after JIA onset. In our study 86% of the patients with JIA developed uveitis in the first 4 years of follow-up, which means that some patients in the non-uveitis group with only 4 years of follow-up might still develop uveitis in the future.

In conclusion, elevated ESR appears to be a predictor for the occurrence of uveitis in patients with JIA. Since ESR is already routinely tested in patients with recently diagnosed arthritis, its use as a biomarker can easily be implemented in daily practice.

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Chapter 3

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

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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
Two cohorts of JIA patients with ophthalmological follow-up were genotyped and then imputed 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. We 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 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 the young (<7 years old), uveitis dramatically impacts quality of life of both children and adults.[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, 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] Nonetheless, severe ocular complications may already be present at the time of a uveitis diagnosis. Females comprise the majority of JIA patients with a female to male ratio of 2:1. The extent to which biological risk for JIA-uveitis is sexually dimorphic, however, is not known.[7]

Both JIA and uveitis are multifactorial autoimmune disorders with a genetic predisposition.[2,8] Both conditions are complex and driven by biological and environmental factors.[2,8] Genome-wide association studies in JIA (regardless of uveitis status) have revealed a number of loci associated to the disease which collectively explain ~20% of the phenotypic variation.[9-11] A number of HLA alleles have also been identified as increasing risk of JIA-uveitis compared to controls.[12] However, genome-wide genetic markers that distinguish JIA without uveitis from JIA-uveitis remain elusive.

Here, we performed genotyping and MHC imputation 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 our analysis in two phases that we then jointly analyzed to improve power for locus discovery (**Table 1**, and **Supplementary Methods**).[13] The study samples were collected in two phases: (a) from a Dutch cohort, and (b) from a cohort of samples collected in Germany, Belgium and Switzerland. For simplicity, we will refer to these as the Phase 1 and Phase 2 sample sets, respectively. After sample- and variant-level quality control was complete (**Supplementary Methods**), the study sample comprised 192 JIA-uveitis samples, 330 non-uveitis JIA samples, and 394 population-level controls (**Table 1**).

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 (36)	247 (64)
Phase 2	192	77 (40)	115 (60)
Total	576	214 (37)	362 (63)
Samples, post-QC			
Phase 1	357	126 (35)	231 (65)
Phase 2	165	66 (40)	99 (60)
Total	522	192 (37)	330 (63)
ANA status			
ANA+	304	150 (49)	154 (51)
ANA-	182	32 (18)	150 (82)
Data unavailable	36	10	26
Sex			
Female	364	136 (37)	228 (63)
Male	158	56 (65)	102 (35)
JIA category			
Oligo-persistent	214	101 (47)	113 (53)
Oligo-extended	98	36 (37)	62 (63)
Poly RFneg	169	46 (27)	123 (73)
Other*	41	9 (22)	32 (78)

* 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)

Genome-wide association testing

We performed genome-wide association testing across the autosomal genome, using a logistic regression framework assuming an additive model in PLINK 1.9,[14,15] correcting for sex and the first two principal components (**Supplementary Figures 1 and 2**). Our data enabled three possible comparison groups: (a) JIA cases and population-level controls, (b) JIA-uveitis cases and population-level controls, and (c) JIA-uveitis cases and non-uveitis JIA. As the goal of our analysis was to investigate similarities and differences in the genetic architecture of JIA and uveitis, and because far larger GWAS of JIA and population-level controls have already been performed [16], we performed the first two genome-wide association studies (GWAS) as a means of data quality control only (**Supplementary Figure 3**) and account for them when considering multiple testing burden. We focus here on the results of the last comparison: JIA-associated uveitis cases and non-uveitis JIA samples.

We performed GWAS in Phase 1 and Phase 2 and then combined them in an inverse variance-weighted fixed effects meta-analysis in METAL[17] for a combined analysis of 330 non-uveitis JIA samples and 192 uveitis cases. In the genome-wide scan, the single signal achieving genome-wide significance ($p < 2 \times 10^{-8}$, after adjusting for three phenotype comparisons) resided in the MHC (**Supplementary Figures 1 and 2**).

Association testing and fine-mapping in the MHC

To identify the amino acids or HLA types driving the genome-wide association signal, we performed a mega-analysis across the imputed MHC data. We merged imputation dosages from Phase 1 and Phase 2, and then used PLINK 1.9[14] to perform logistic regression, assuming an additive model and correcting for the top 5 principal components, 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] odds ratios derived from mega-analysis and meta-analysis of Phase 1 and Phase 2 were highly concordant (Pearson's r = 0.95, **Supplementary Methods** and **Supplementary Figure 4**). The mega-analysis allows for the additional advantage of allowing for interaction testing and conditional analysis on any associated variants across the full dataset.

Of the SNPs, amino acids, and classical alleles tested in the MHC, we observed the strongest association for the presence of either serine (S) or aspartic acid (D) at position 11 in *HLA-DRB1* (OR = 2.59 [95% CI: 1.92 - 3.50], $p = 4.80 \times 10^{-10}$; **Figure 1, Table 2**). To identify which of the possible residues at position 11 explain 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, principal components and genotyping batch (likelihood ratio test $p = 1.5 \times 10^{-9}$). 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 conditioned the top association signal first on serine and then on aspartic acid at position 11, by including the dosages of the respective variant as an additional covariate in the logistic regression model (**Figure 1, Table 2, Supplementary Table 2**). After conditioning on serine at position 11, the association signal at position 11 dropped substantially (p = 0.069), while conditioning on aspartic acid at position 11 left the association 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 at serine-11 in *HLA-DRB1* was consistent in direction of effect and magnitude of association 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 x 10^{-5}), indicating that both phases of the study contributed to the joint association signal (in the mega-analysis.[13]

Serine at position 11 in *HLA-DRB1* is positioned in the middle of what is known as the YST-motif in *HLA-DRB1*;[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., LD = 1 between all residue pairs). Additionally, the residues in the YST-motif are also in perfect LD with a fourth residue, presence of serine (S) or glycine (G) at position 13 in *HLA-DRB1* (OR = 2.47 [95% CI: 1.84 - 3.32, p = 1.44 x 10^{-9} in the MHC analysis). Thus, all four amino acid configurations show statistically identical association to JIA-uveitis (**Table 2**). To further decipher the specific residue(s) 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 which of the models, if either, best fits the data. We found that the S (or Y or T) amino acids best fit the data (p = 1.48 x 10^{-10}, compared to a model containing only PCs and sex as independent variables). Including the S/G residue at position 13 as well as the YST motif only modestly improved the fit of the model (likelihood ratio test p = 0.043, compared to a model containing serine at position 11 only); neither the S nor G residues alone at position 13 improved the model (p = 0.70 for both tests).

A recent MHC fine-mapping study of oligoarticular and RF-negative polyarticular JIA mapped the primary disease association signal to the G residue at position 13 in *HLA-DRB1*, and additionally identified an independent risk for serine at the same position. Analyzing all JIA patients (with and without uveitis), we also observed an association at glycine-13 (**Supplementary Table 3**). We sought to disentangle the previously-described association signal in all JIA (residues S or G at position 13) from the JIA-uveitis signal (residue S at position 11) observed here. We adjusted for either G or S at position 13 in the JIA-uveitis vs. non-uveitis JIA analysis and found the signal at serine-11 to be only moderately affected (serine-11 adjusted for glycine-13: OR = 2.41 [95% CI: 1.75 - 3.32], p = 6.49 x 10^{-8}; adjusted for serine-13; OR = 2.61 [95% CI: 1.74 - 3.93], p = 3.83 x 10^{-6}). These results indicate that the association signal in uveitis at serine-11 (or tyrosine-10 or threonine-12) cannot be fully explained by the JIA-associated amino acids in position 13 in *HLA-DRB1*.

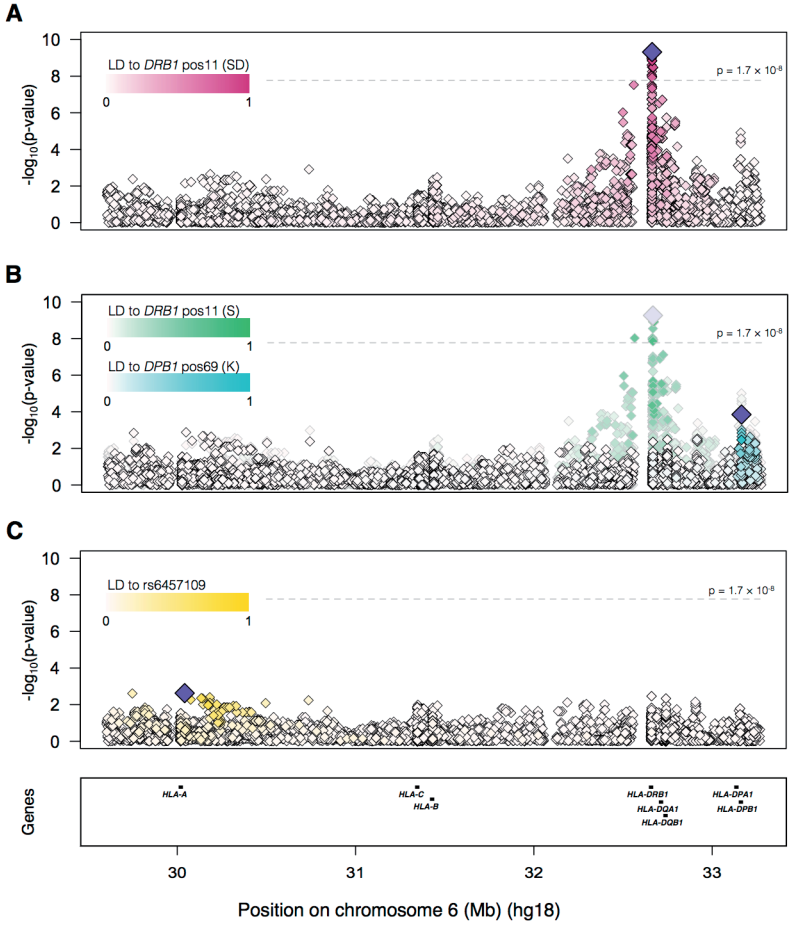

Figure 1. Association and conditional testing in HLA-DRB1. *A. Initial association testing in the MHC revealed a genome-wide significant signal at HLA-DRB1 position 11 (presence of serine (S) or aspartic acid (D), purple diamond). B. Conditioning on the presence of aspartic acid at position 11 left the 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).*

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.

Sexual dimorphism in JIA-uveitis

Since uveitis is epidemiologically known to be more prevalent in females with oligoarticular JIA,[22] we wanted to formally test if the primary signal in uveitis showed evidence of sexual dimorphism in our sample. In testing female samples only, 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 such an association in the male samples (OR = 1.41 [95% CI: 0.86 - 2.31], p = 0.177; **Table 2** and **Supplementary Figure 5**). 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 (T), OR = 0.30 [95% CI: 0.20 - 0.41], p = 3.50 × 10^{-10}; **Table 2**), this position is in nearly perfect LD (r^2=0.98) with tyrosine (Y) at position 10, serine (S) at position 11, threonine (T) at position 12 (the YST-motif), and position 13 in *HLA-DRB1* (**Supplementary Figure 6**), and consequently yield a nearly identical association (**Table 2**).

To further explore the potential for a sex-specific effect at *HLA-DRB1* position 11, we ran the association test at serine-11 across all samples, including an interaction term between sex and the imputed dosage at 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 serine-11 signal in *HLA-DRB1* was indeed a sex-specific effect.

In Silico Peptide Binding to HLA-DRβ1

Polymorphisms in the beta chain (HLA-DRB1) specify the peptide binding preference of HLA-DR. The YST-motif (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 developing uveitis. To explore if the presence of serine-11 affects peptide-MHCII interactions, we compared the predicted binding affinity for 13 common HLA-DRB1 allotypes (representing 79% of *DRB1* alleles in cases) using a large panel of >80,000 peptides based on human iris proteome data (**Supplementary Methods** and **Supplementary Table 4**) using 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.

To compare the 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 7**). 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 x 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,(25) we selected for peptides with an (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 Methods**). 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**).

Previously-described associations to JIA and uveitis

A number of HLA types and amino acid positions have been demonstrated to be associated to JIA and uveitis.[16,24] We looked up 10 previously-associated HLA types and amino acids in our GWAS data (**Supplementary Table 6** and **Supplementary Figure 8**).

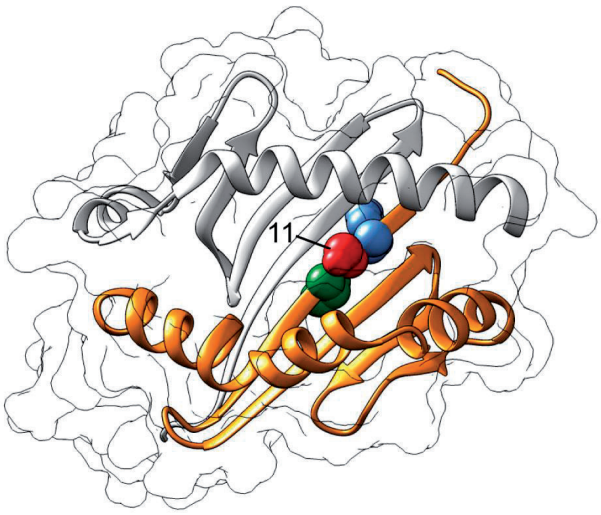

Figure 2. Three-dimensional ribbon model for HLA-DR *(Protein Data Bank entry: 3pdo). The molecule is positioned to provide a view from the top of the peptide-binding groove. The beta-chain (DRß) is highlighted in orange. Amino acid serine at position 11 (red) is located in the bottom center of the peptide-binding 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. 3D structure was produced using UCSF Chimera (60).*

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 patients with JIA. This association indicates that, though JIA with uveitis and JIA without uveitis share clinical features and genetic risk factors (and in particular, HLA-specific genetic risk factors)[24], there is genetic architecture specific to uveitis.[25]

Association testing initially revealed either serine or aspartic acid at position 11 in *HLA-DRB1* as the most-associated variant, a signal we determined was primarily driven by serine. However, the perfect linkage disequilibrium between serine, tyrosine (position 10), and threonine (position 12) makes it impossible to disentangle the three amino acids in a statistical framework; a subset of the amino acids, or all three (that is, the YST-motif) may be key to disease onset. Importantly, though all three residues are located 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). Some JIA patients, including those with uveitis, might be categorized as seronegative RA by the time they reach adulthood, and seronegative RA is considered to genetically mirror the uveitis-prone JIA categories.[16] 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).[26]

Sex-specific analyses in the MHC further revealed the *HLA-DRB1* signal was unique to female samples. The reduced number of cases (N = 56 male JIA-uveitis cases and N = 102 male non-uveitis JIA controls) and therefore reduced power in the male-only analysis may explain the absence of a signal at serine-11. However, we had 98.5% power to detect an association of $p < 0.05$ 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 potentially more subtle effects in males (OR < 2), larger case collections will be necessary to improve power. Nonetheless, the interaction test of sex and serine-11 provides compelling evidence for a sexually-dimorphic signal in *HLA-DRB1*.

In-depth data on sex-specific epidemiology of uveitis associated with JIA are sparse, however, JIA-uveitis has been reported to occur more frequently in females.[22] This genotype-by-sex interaction informs an exciting field of future research that aims to elucidate how the uveitis risk mechanisms underlying the *HLA-DRB1* signal may be mediated by sex-specific factors, such as hormone regulation. Further, the sex-specific pattern of genetic association may help to resolve previously reported evidence of sexual dimorphism with respect to severity or disease course in children with JIA-uveitis.[27] 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.[28]

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;[29] 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 the regulation of T helper cells. Although the large panel of putative antigens that we tested was by no means exhaustive (**Supplementary Table 4**), the experiment demonstrated that the presence of a serine-11 motif in *HLA-DRB1* is accompanied by distinguished changes in binding affinity in the

resulting protein (**Supplementary Table 5** and **Supplementary Figures 7**), 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 significantly increase the likelihood of downstream immune responses (e.g., ANA) to the eye; ANA-positivity is modestly correlated with presence of serine-11 in the cases studied here (Pearson's r= 0.21, $P = 1.0 \times 10^{-6}$). 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. Genes outside the MHC have been implicated in JIA-uveitis, including a polymorphism in *VTCN1*[30] and variants near the immune genes *TRAF1* and *C5*.[31] Only a handful of candidate gene studies[2,32] and HLA-specific analyses[24] have investigated uveitis as a phenotype separate from all JIA. A recent GWAS identified *HLA-DRB1*1501* as a risk factor for uveitis[33] and recent studies in JIA-uveitis point toward a role for infiltrated plasma cells,[34] T helper cells[2], and changes in the ocular fluid microenvironment.[25,35,36] Given our sample size, we were well powered to detect common (frequency >10%) and highly-penetrant (odds ratio > 3, **Supplementary Figure 9**) variation that associates with increased uveitis risk compared to JIA individuals without uveitis. However, we are underpowered to identify common (frequency 1-10%), modestly-penetrant variation (odds ratio 1.05 - 1.5); such variation has been the hallmark genetic feature of complex phenotypes,[37,38] and future studies will need to interrogate larger samples in order to identify additional genetic signals both within and outside of the MHC influencing disease risk.

Risk factors such as young age and presence of ANA, assumed to reflect aberrant immune activation,[39] indicate patients potentially at higher risk of uveitis but are in no way predictive of disease outcome. Using these risk factors, JIA patients are regularly screened (~4 times a year) 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 would make diagnosis significantly simpler and prevent unnecessary ophthalmologic screening in JIA patients with low susceptibility for uveitis.[4,27] Intriguingly, after deeper examination of the phenotypic data, we found that 99% of female uveitis cases in our study carry at least one copy of the variant coding for serine-11. The only female uveitis case who did not carry 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. Unraveling the pathogenesis of the two diseases will improve understanding of the key pathways that

trigger disease. 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.

MATERIAL AND METHODS

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)).[40,41] All patients were screened by an ophthalmologist according to the guidelines of the Academy of Pediatrics and patients with no clinical signs of uveitis had an ophthalmologic follow-up of at least 4 years after onset of JIA.[6] Patients with at least 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 JIA patients with and without uveitis from Phase 1 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 based in the Netherlands). Samples from Phase 2 were collected within 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.[42]

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

The Phase 1 and Phase 2 data were phased separately using SHAPEIT2.[43] As the sample size was >100 samples, prephasing was run without a reference panel, per the SHAPEIT2 recommendations.[43]

Following prephasing, we imputed all samples using the imputation reference panel constructed through whole-genome sequencing of 2,504 samples in 1000 Genomes Project (Phase 3).[44] We imputed the prephased haplotypes using IMPUTE2.[45,46]

To impute amino acids and HLA alleles in the MHC, we used the SNP2HLA pipeline [47]. The panel includes SNPs and amino acids in the MHC, as well as HLA types from the Class I (*HLA-A*, *HLA-B*, and *HLA-C*) and Class II (*HLA-DRB1*, *HLA-DQA1*, *HLA-DQB1*, *HLA-DPA1*, and *HLA-DPB1*) HLA genes. HLA types are imputed to 2- and 4-digit resolution. The SNP2HLA pipeline uses BEAGLE [48] to phase the data and then impute. The full details of prephasing and imputation can be found in the Supplemental Methods.

Genome-wide association testing

GWAS were performed using PLINK 1.9[14] using an additive logistic regression model, correcting for the top two principal components and sex. Data were then meta-analyzed using METAL.[17] 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[14] 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). 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, thus, 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. Heatmaps were created 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.

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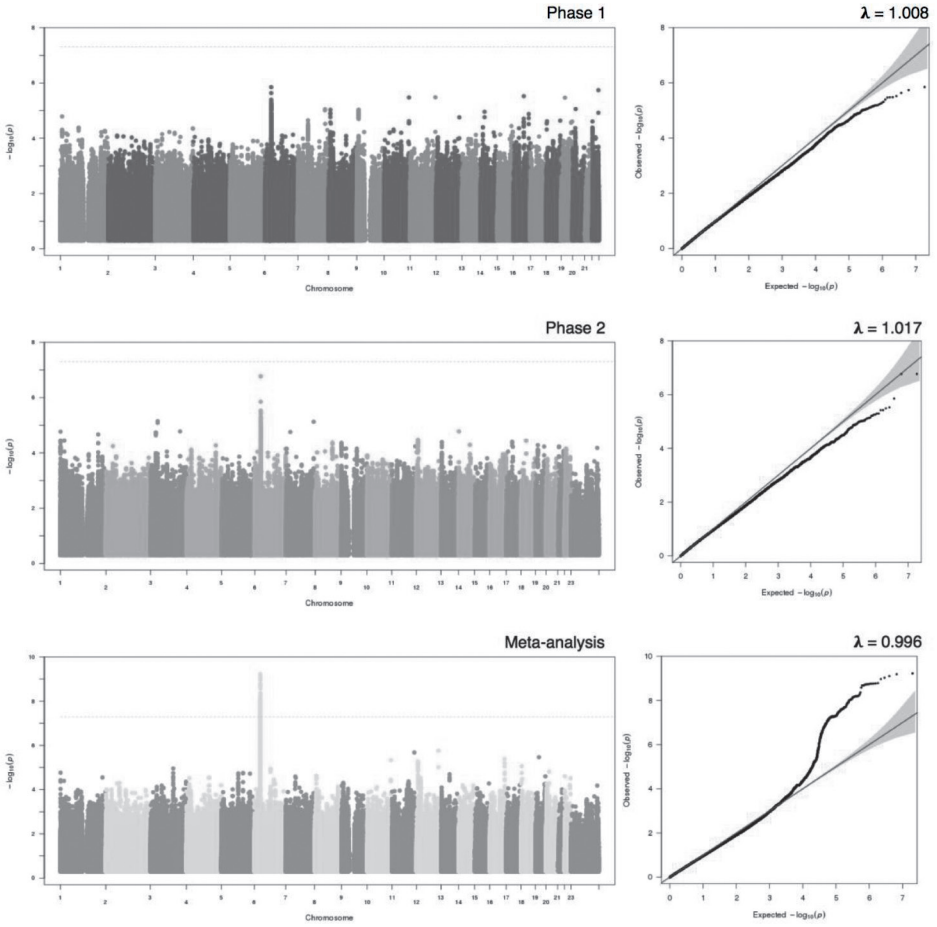
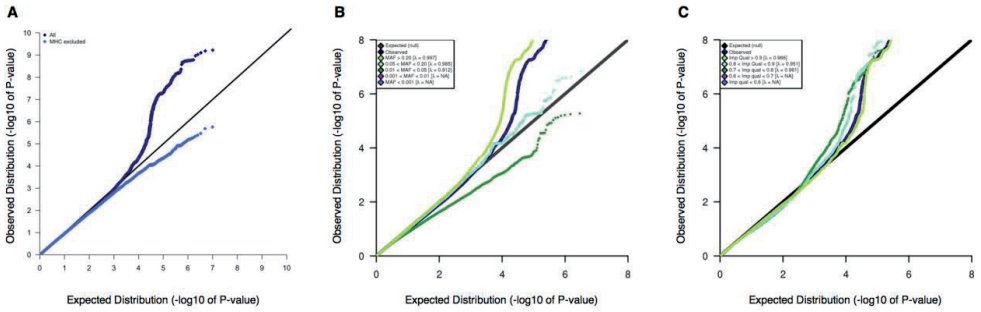
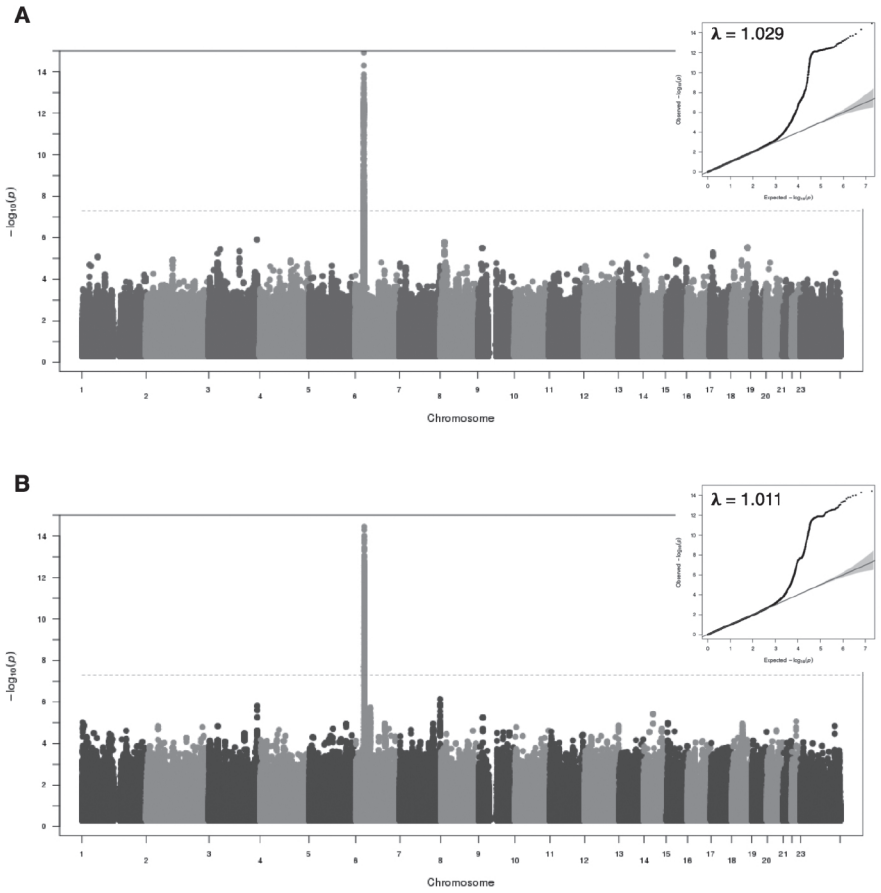
SUPPLEMENTARY INFO



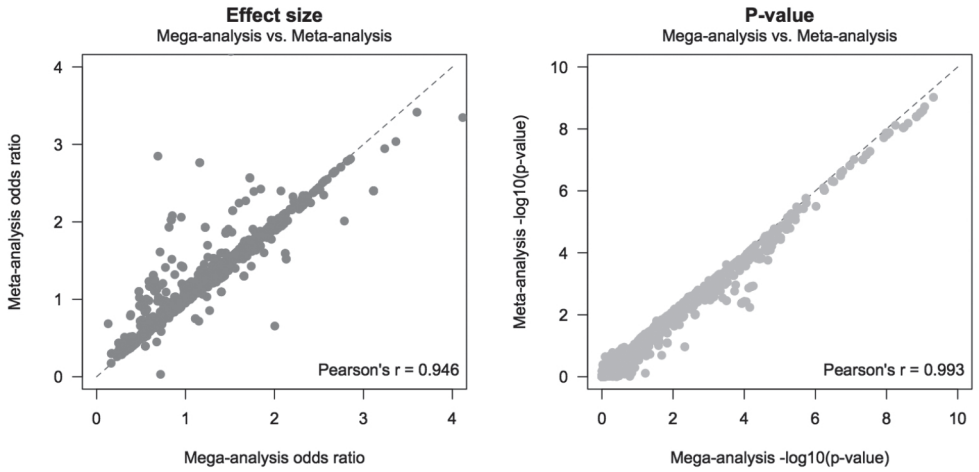
Supplementary Figure 1. Manhattan plots and quantile-quantile (QQ) plots for Phase 1, Phase 2 and meta-analysis of genome-wide association testing in uveitis versus non-uveitis JIA. *Genome-wide significance ($p < 5 \times 10^{-8}$) is indicated as a dotted line in the Manhattan plots. The genome-wide inflation factor lambda (λ) for each analysis is indicated above the QQ plots. After meta-analysis, only a locus in the major histocompatibility complex (MHC) achieved genome-wide significance.*



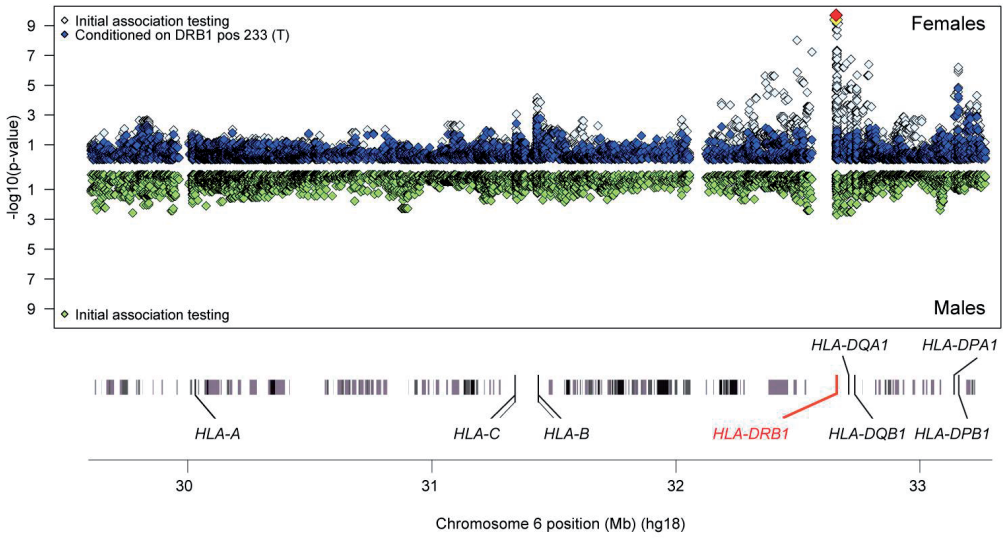
Supplementary Figure 2. Stratified QQ plots of the final genome-wide meta-analysis of non-uveitis JIA versus uveitis individuals. *QQ plots show the overall meta-analysis (A), as well as stratified by frequency (B) and by imputation quality (info) score (C). A. The QQ plot is shown for the entire meta-analysis, both before (navy blue) and after (lighter blue) removal of the MHC. B. The QQ and genomic inflation factor (lambda) is shown for various frequency stratifications. C. The QQ and genomic inflation factor (lambda) is shown for various imputation quality stratifications.*



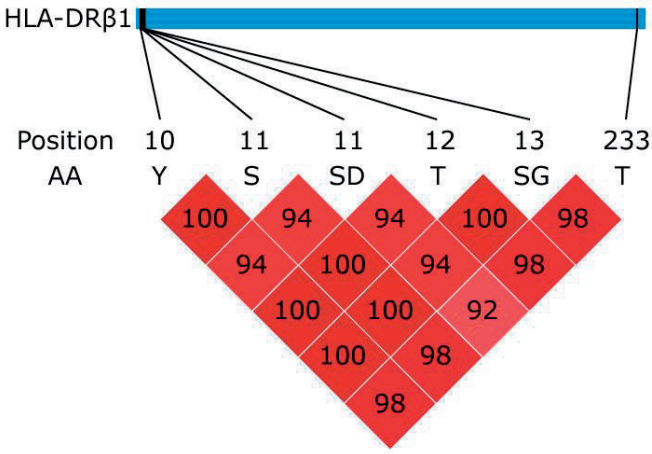
Supplementary Figure 3. Genome-wide association testing in non-uveitis JIA, uveitis, and population-level controls. *Phase 1 of our study contained (1) population-level controls (N = 394), (2) individuals with JIA but not with uveitis (N = 357), and (3) individuals with uveitis in JIA (N = 126). For quality control purposes, we performed GWAS in population-level controls vs. all JIA and population-level controls versus uveitis in JIA. A. Genome-wide association study (GWAS) of JIA samples versus population-level controls. The Manhattan and QQ plots show GWAS results generated after genotype quality control and imputation using the 1000 Genomes Phase 3 reference panel was complete (see Materials and Methods). Variants included in the GWAS have minor allele frequency (MAF) > 1% and imputation quality (info) score > 0.7. B. GWAS of uveitis versus population-level controls. Cleaned and imputed data (as in A) is shown. Variants included in the GWAS have MAF > 1% and imputation info score > 0.7.*



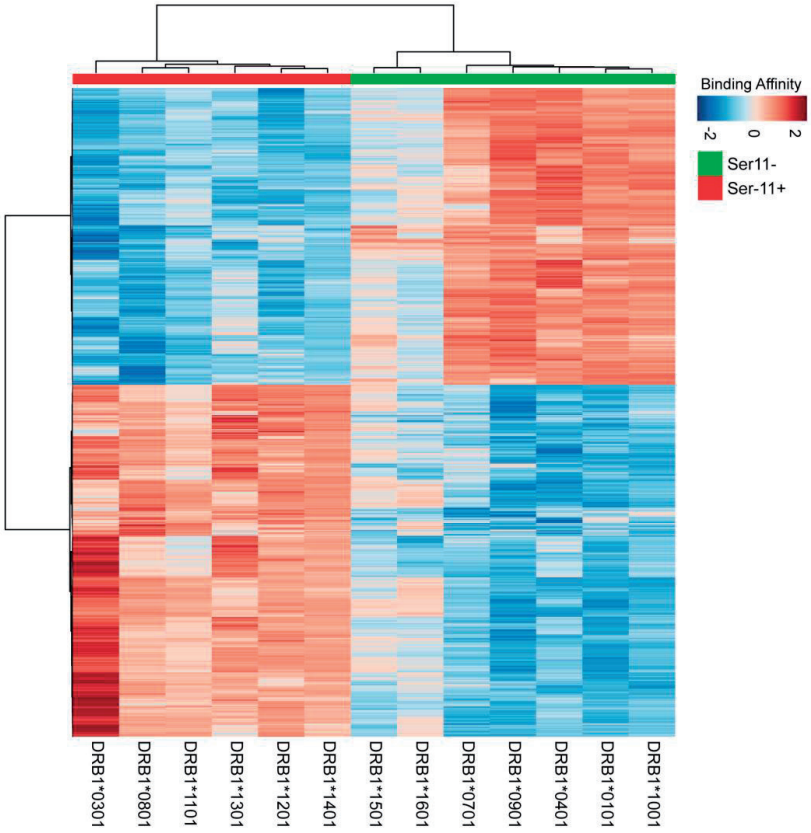
Supplementary Figure 4. Concordance of mega-analysis and meta-analysis in the MHC. *As discussed in the main article, inverse variance-weighted fixed effects meta-analysis and mega-analysis (the joint association testing of two datasets, including a covariate to indicate the studies comprising the mega-analysis) are theoretically and empirically highly concordant in their results. The meta-analysis and mega-analysis of variation in the MHC demonstrate highly concordant results. The effect sizes (i.e., odds ratios) are highly concordant (Pearson's r = 0.946, left panel), as are the -log$_{10}$(p-values) (Pearson's r = 0.993, right panel).*



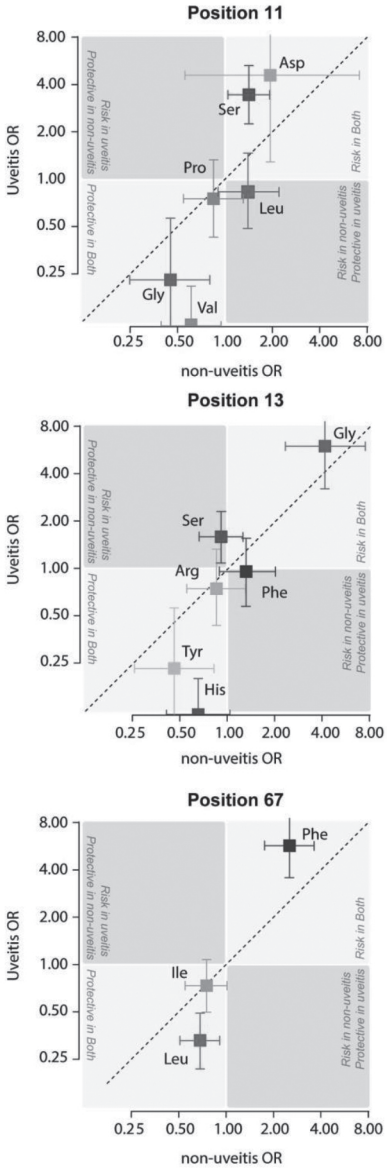
Supplementary Figure 5. Sex-specific association testing and conditional testing in HLA-DRB1. *Initial sex-specific association testing in the MHC revealed a female-specific genome-wide significant signal at HLA-DRB1 position 233 (presence of threonine (T), red diamond), which is in near perfect LD (r^2=0.98) with the associated variant serine at position 11 (yellow diamond), discovered in the joint analysis of males and females. Conditioning on the presence of threonine at position 233 (dark blue diamonds) mitigated this female-specific association signal. Similar testing for males revealed no associations exceeding the threshold for genome-wide significance (green diamonds).*



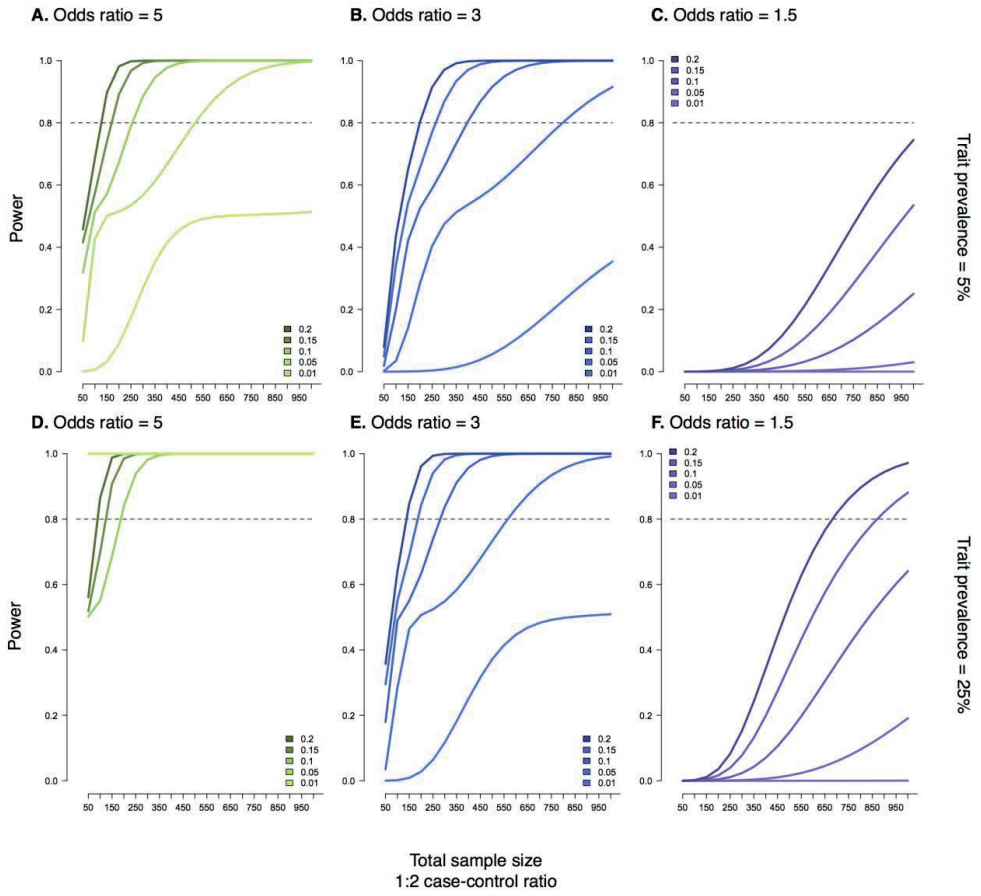
Supplementary Figure 6. The Linkage Disequilibrium (LD) structure for the leading amino acids in HLA-DRB1 associated with JIA-associated uveitis in females. *The amino acid residues and their relative positions are indicated in the HLA-DRβ1 protein sequence (in blue). The r^2 measure of LD (x100) is indicated and accompanied by color-coded values of the pairwise LD measurements range from white (low) to red (high).*



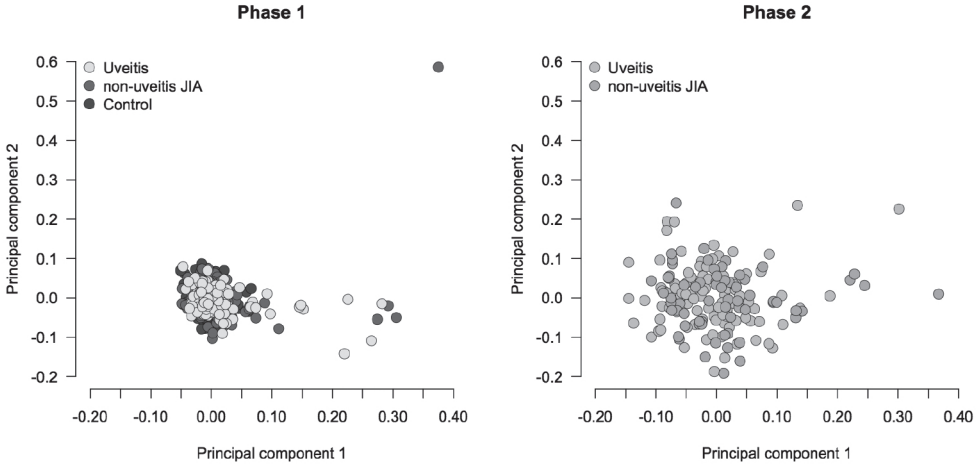
Supplementary Figure 7. Unsupervised hierarchical clustering of predicted peptide binding affinities to common HLA-DRB1 alleles. *The heatmap displays top 1,674 peptides (top 2% of all putative 84,686 peptides) with the highest variance in predicted binding affinities (see Materials and Methods in the main manuscript) for 13 common HLA-DRB1 alleles (representing 79% of all HLA-DRB1 alleles in the JIA cases). Unsupervised Hierarchical clustering on quantile normalized, log-transformed, auto-scaled data was performed based on the Euclidian distance measure and the Ward's linkage method using the MetaboAnalyst server. Two major clusters can be discriminated based on overall peptide binding preference, which follow the distribution of serine-11 (in red) among the HLA-DRB1 alleles tested. Heatmap colors represent the binding affinity for each peptide to the DRß1 proteins from low (blue) to high (red). Dendrograms indicating the clustering relationships are shown to the left and above the heat map.*



Supplementary Figure 8. Association results for amino acid residues in HLA-DRB1 for JIA with and without uveitis. *Odds ratios (OR) and 95% confidence interval (CI) for amino acid residues at position 11, 13 and 67 of HLA-DRB1 comparing JIA-uveitis with healthy controls (y-axis), and comparing JIA non-uveitis with healthy controls (x-axis).*



Supplementary Figure 9. Power for locus discovery under various genetic architectures. *Our analysis contained 192 uveitis cases and 330 non-uveitis JIA cases (an approximately 1:2 case-control ratio). Here, we plot power to discover a genetic variant at genome-wide significance ($p < 5 \times 10^{-8}$) for variants ranging in frequency from 1% - 20% (shown in legends). We assume a trait prevalence of 5% (panels A - C) and 25% (panels D - F) to represent the range of potential prevalences of uveitis in JIA. We provide models for high-penetrant variants (odds ratios of 5 and 3) and modest-effect penetrant variants (odds ratio of 1.5) to demonstrate that, while our study was well powered to detect highly-penetrant variants, we were underpowered to detect modest-effect less-frequent variants (which are the variants likely to confer disease risk, in the scenario that uveitis is a complex trait).*



Supplementary Figure 10. Principal component analysis. *The mega-analysis in the MHC was corrected for principal components calculated in the Phase 1 and Phase 2 data (as well as a covariate indicating whether the sample was genotyped as part of Phase 1 or Phase 2). Principal component analysis (PCA) was performed on SNPs with minor allele frequency > 5%, genotype missingness < 0.1%, and linkage disequilibrium pruned at an r2 threshold of 0.2. Additionally, the MHC, lactase locus on chromosome 2, and inversions on chromosomes 8 and 17 were excluded from PCA analysis. PCs 1 and 2 are plotted here. The analysis indicated a homogenous distribution of samples with similar genetic ancestry across uveitis, non-uveitis JIA, and population-level control samples.*

Supplementary Table 1. Summary of Phase 1 and Phase 2 genotyping and imputation data. Sample counts (after quality control) are reported for Phase 1, Phase 2, and the joint analysis. Breakdowns for JIA (uveitis and non-uveitis) are shown. Genotyped SNPs (after quality control) are also reported. Imputed SNPs available for association testing after quality control (minor allele frequency ≥ 1% and info score > 0.7 for genome-wide imputation; minor allele frequency ≥ 1% for MHC imputation) are also provided.

	Phase 1	**Phase 2**	**Joint analysis**
Samples			
Total samples	751	165	916
Total JIA	357	165	522
Uveitis	126	66	192
Non-uveitis JIA	231	99	330
Population-level (controls)	394	--	394
Variants			
Genotyped SNPs	574,222	619,388	625,661
Imputed SNPs (% with info > 0.9)			
Genome-wide	9,250,718 (82.5)	9,300,016 (77.4)	10,043,928
MHC	7,987 (92.3)	7,920 (88.2)	7,989

Supplementary Table 2. Association results for HLA alleles with frequency >0.01 in JIA-uveitis cases versus JIA non-uveitis controls. For each amino acid, the frequency in cases and controls, the imputation-score (INFO; a score between 0 and 1, a higher value means a better imputation of that amino acid), odds ratio (OR), 95%-confidence interval (95%-CI) and p-values are given.

Classical Allele	Frequency		INFO	OR	95% CI	P-value
	Cases	Controls				
HLA-A*01	0.122	0.153	0.99	0.76	(0.52-1.12)	0.165
HLA-A*0101	0.122	0.153	0.99	0.77	(0.52-1.12)	0.169
HLA-A*02	0.388	0.347	0.92	1.25	(0.95-1.65)	0.113
HLA-A*0201	0.378	0.339	0.89	1.26	(0.95-1.67)	0.116
HLA-A*0205	0.010	0.002	0.95	5.72	(0.68-48.2)	0.109
HLA-A*03	0.112	0.130	1.02	0.83	(0.56-1.23)	0.363
HLA-A*0301	0.112	0.130	1.02	0.83	(0.56-1.23)	0.364
HLA-A*11	0.073	0.068	0.95	1.12	(0.68-1.86)	0.654
HLA-A*1101	0.073	0.068	0.95	1.12	(0.68-1.87)	0.651
HLA-A*24	0.104	0.109	1.04	0.92	(0.61-1.39)	0.696
HLA-A*2402	0.104	0.109	1.01	0.90	(0.59-1.38)	0.636
HLA-A*25	0.016	0.012	0.84	1.16	(0.35-3.8)	0.807
HLA-A*2501	0.016	0.012	0.84	1.16	(0.35-3.8)	0.807
HLA-A*26	0.036	0.026	0.97	1.46	(0.7-3.02)	0.312
HLA-A*2601	0.036	0.026	0.97	1.46	(0.7-3.03)	0.313
HLA-A*29	0.010	0.012	0.97	0.81	(0.24-2.8)	0.744
HLA-A*30	0.010	0.014	1.12	0.76	(0.24-2.37)	0.631
HLA-A*3001	0.010	0.008	0.97	1.36	(0.35-5.25)	0.653
HLA-A*31	0.023	0.021	0.97	1.14	(0.48-2.71)	0.772
HLA-A*3101	0.023	0.021	0.97	1.14	(0.48-2.73)	0.762
HLA-A*32	0.042	0.030	0.97	1.35	(0.68-2.68)	0.399
HLA-A*3201	0.042	0.030	0.97	1.35	(0.68-2.68)	0.399
HLA-A*68	0.042	0.056	1.02	0.69	(0.38-1.26)	0.230
HLA-A*6801	0.039	0.048	1.03	0.76	(0.41-1.43)	0.400
HLA-C*01	0.034	0.039	0.96	0.83	(0.41-1.69)	0.609
HLA-C*0102	0.034	0.039	0.96	0.83	(0.41-1.69)	0.609
HLA-C*02	0.068	0.062	0.93	1.03	(0.6-1.76)	0.925
HLA-C*0202	0.068	0.062	0.93	1.03	(0.6-1.76)	0.925
HLA-C*03	0.180	0.161	0.94	1.29	(0.9-1.84)	0.163
HLA-C*0303	0.060	0.061	1.06	1.05	(0.63-1.77)	0.849
HLA-C*0304	0.117	0.098	0.90	1.40	(0.9-2.17)	0.132
HLA-C*04	0.120	0.144	1.06	0.79	(0.54-1.15)	0.211
HLA-C*0401	0.120	0.144	1.06	0.79	(0.54-1.15)	0.211
HLA-C*05	0.091	0.059	0.95	1.68	(1.02-2.77)	0.041
HLA-C*0501	0.091	0.059	0.95	1.68	(1.02-2.77)	0.041
HLA-C*06	0.042	0.059	1.02	0.69	(0.38-1.26)	0.229
HLA-C*0602	0.042	0.059	1.02	0.69	(0.38-1.26)	0.229
HLA-C*07	0.315	0.320	1.01	0.99	(0.75-1.29)	0.922
HLA-C*0701	0.146	0.159	0.98	0.91	(0.63-1.3)	0.591
HLA-C*0702	0.146	0.141	1.02	1.05	(0.73-1.5)	0.797
HLA-C*0704	0.023	0.020	0.98	1.19	(0.49-2.86)	0.699
HLA-C*08	0.016	0.012	0.98	1.35	(0.45-4.08)	0.594
HLA-C*0802	0.016	0.012	0.98	1.35	(0.45-4.08)	0.595
HLA-C*12	0.076	0.073	0.98	0.98	(0.6-1.61)	0.945
HLA-C*1203	0.073	0.067	0.98	1.05	(0.64-1.74)	0.837
HLA-C*14	0.010	0.009	0.99	1.29	(0.36-4.67)	0.698
HLA-C*1402	0.010	0.009	0.99	1.29	(0.36-4.67)	0.698
HLA-C*15	0.021	0.032	0.97	0.58	(0.24-1.36)	0.208
HLA-C*1502	0.018	0.030	0.95	0.54	(0.22-1.35)	0.190
HLA-C*16	0.023	0.024	0.98	0.95	(0.41-2.21)	0.904

HLA-C*1604	0.010	0.006	0.96	1.52	(0.36-6.45)	0.570
HLA-B*07	0.104	0.105	1.02	1.00	(0.67-1.52)	0.982
HLA-B*0702	0.099	0.103	1.03	0.98	(0.64-1.48)	0.910
HLA-B*08	0.091	0.094	1.01	1.01	(0.65-1.57)	0.948
HLA-B*0801	0.091	0.094	1.01	1.01	(0.65-1.57)	0.948
HLA-B*13	0.013	0.017	0.96	0.92	(0.31-2.75)	0.888
HLA-B*1302	0.013	0.017	0.96	0.92	(0.31-2.75)	0.888
HLA-B*14	0.016	0.014	0.96	1.36	(0.45-4.1)	0.583
HLA-B*1402	0.013	0.012	0.96	1.27	(0.38-4.17)	0.698
HLA-B*15	0.060	0.080	0.99	0.82	(0.49-1.37)	0.441
HLA-B*1501	0.055	0.074	0.93	0.76	(0.44-1.34)	0.344
HLA-B*18	0.052	0.055	0.96	0.95	(0.53-1.71)	0.873
HLA-B*1801	0.052	0.055	0.96	0.95	(0.53-1.71)	0.873
HLA-B*27	0.052	0.070	0.99	0.71	(0.4-1.25)	0.231
HLA-B*2705	0.052	0.068	0.96	0.70	(0.39-1.26)	0.233
HLA-B*35	0.128	0.130	1.03	0.90	(0.62-1.32)	0.599
HLA-B*3501	0.063	0.077	0.90	0.67	(0.38-1.18)	0.165
HLA-B*3502	0.029	0.020	0.99	1.42	(0.61-3.35)	0.419
HLA-B*3503	0.031	0.030	0.96	1.04	(0.49-2.2)	0.911
HLA-B*38	0.049	0.041	0.92	1.27	(0.66-2.45)	0.474
HLA-B*3801	0.047	0.041	0.92	1.27	(0.66-2.45)	0.482
HLA-B*39	0.052	0.053	0.95	1.06	(0.6-1.89)	0.835
HLA-B*3901	0.021	0.024	0.81	1.07	(0.43-2.64)	0.888
HLA-B*3906	0.026	0.030	0.99	0.98	(0.45-2.13)	0.958
HLA-B*40	0.135	0.094	0.91	1.73	(1.14-2.64)	0.011
HLA-B*4001	0.109	0.076	0.92	1.77	(1.12-2.81)	0.015
HLA-B*4002	0.026	0.018	0.95	1.67	(0.68-4.09)	0.264
HLA-B*44	0.138	0.102	0.99	1.48	(1-2.2)	0.051
HLA-B*4402	0.120	0.077	0.93	1.74	(1.11-2.71)	0.015
HLA-B*4403	0.010	0.020	0.95	0.61	(0.19-1.96)	0.409
HLA-B*51	0.044	0.059	0.99	0.78	(0.43-1.4)	0.404
HLA-B*5101	0.044	0.059	0.98	0.78	(0.43-1.41)	0.412
HLA-B*55	0.018	0.017	0.91	1.08	(0.39-2.96)	0.885
HLA-B*5501	0.018	0.017	0.91	1.08	(0.39-2.96)	0.886
HLA-B*57	0.010	0.024	0.98	0.39	(0.13-1.2)	0.102
HLA-B*5701	0.010	0.024	0.98	0.41	(0.13-1.25)	0.116
HLA-DRB1*01	0.073	0.146	0.94	0.44	(0.28-0.7)	5.53E-04
HLA-DRB1*0101	0.065	0.139	0.92	0.38	(0.23-0.63)	1.34E-04
HLA-DRB1*03	0.083	0.077	0.99	1.09	(0.68-1.74)	0.724
HLA-DRB1*0301	0.081	0.077	0.99	1.06	(0.66-1.71)	0.796
HLA-DRB1*04	0.023	0.089	1.04	0.24	(0.11-0.49)	1.15E-04
HLA-DRB1*0401	0.016	0.058	0.96	0.16	(0.06-0.48)	8.85E-04
HLA-DRB1*07	0.023	0.055	0.95	0.39	(0.18-0.84)	0.016
HLA-DRB1*0701	0.023	0.055	0.95	0.39	(0.18-0.84)	0.016
HLA-DRB1*08	0.195	0.121	0.85	2.02	(1.37-2.97)	3.62E-04
HLA-DRB1*0801	0.185	0.109	0.85	2.12	(1.42-3.16)	2.30E-04
HLA-DRB1*09	0.023	0.018	0.98	1.33	(0.55-3.25)	0.528
HLA-DRB1*0901	0.023	0.018	0.98	1.33	(0.55-3.25)	0.528
HLA-DRB1*11	0.240	0.152	0.96	1.78	(1.27-2.51)	9.40E-04
HLA-DRB1*1101	0.112	0.074	0.75	2.07	(1.23-3.46)	5.82E-03
HLA-DRB1*1103	0.018	0.015	0.62	0.57	(0.14-2.22)	0.415
HLA-DRB1*1104	0.104	0.053	0.82	2.32	(1.34-4.02)	2.72E-03
HLA-DRB1*12	0.016	0.027	0.95	0.53	(0.2-1.39)	0.197
HLA-DRB1*1201	0.016	0.027	0.95	0.53	(0.2-1.39)	0.198
HLA-DRB1*13	0.193	0.153	0.92	1.39	(0.98-1.97)	0.062
HLA-DRB1*1301	0.146	0.092	0.94	1.76	(1.17-2.65)	6.30E-03
HLA-DRB1*1302	0.039	0.056	0.95	0.69	(0.37-1.31)	0.257
HLA-DRB1*14	0.042	0.036	0.98	1.16	(0.6-2.27)	0.655
HLA-DRB1*1401	0.036	0.032	0.88	1.03	(0.48-2.25)	0.933

HLA-DRB1*15	0.057	0.089	1.01	0.65	(0.39-1.08)	0.093
HLA-DRB1*1501	0.057	0.083	1.03	0.71	(0.43-1.19)	0.192
HLA-DRB1*16	0.029	0.027	0.95	1.02	(0.46-2.29)	0.956
HLA-DRB1*1601	0.023	0.026	0.94	0.81	(0.33-1.95)	0.636
HLA-DQA1*01	0.393	0.453	1.02	0.80	(0.62-1.04)	0.094
HLA-DQA1*0101	0.117	0.186	0.94	0.56	(0.38-0.82)	3.29E-03
HLA-DQA1*0102	0.128	0.168	0.98	0.74	(0.51-1.07)	0.110
HLA-DQA1*0103	0.148	0.098	0.96	1.64	(1.11-2.44)	0.014
HLA-DQA1*02	0.023	0.055	0.95	0.39	(0.18-0.84)	0.017
HLA-DQA1*0201	0.023	0.055	0.95	0.39	(0.18-0.84)	0.017
HLA-DQA1*03	0.047	0.111	1.02	0.40	(0.23-0.69)	9.06E-04
HLA-DQA1*0301	0.047	0.111	1.02	0.40	(0.23-0.69)	9.06E-04
HLA-DQA1*04	0.185	0.114	0.85	2.02	(1.36-2.99)	4.72E-04
HLA-DQA1*0401	0.185	0.114	0.85	2.02	(1.36-2.99)	4.72E-04
HLA-DQA1*05	0.346	0.264	0.97	1.46	(1.1-1.95)	9.30E-03
HLA-DQA1*0501	0.346	0.264	0.97	1.46	(1.1-1.95)	9.30E-03
HLA-DQB1*02	0.094	0.114	0.96	0.81	(0.53-1.25)	0.348
HLA-DQB1*0201	0.081	0.080	1.01	1.02	(0.64-1.63)	0.928
HLA-DQB1*0202	0.013	0.033	0.97	0.38	(0.14-1.03)	0.057
HLA-DQB1*03	0.328	0.320	0.97	1.00	(0.75-1.32)	0.989
HLA-DQB1*0301	0.271	0.218	0.98	1.28	(0.95-1.74)	0.107
HLA-DQB1*0302	0.023	0.065	1.03	0.35	(0.16-0.73)	5.36E-03
HLA-DQB1*0303	0.034	0.036	0.96	0.92	(0.45-1.85)	0.806
HLA-DQB1*04	0.185	0.114	0.85	2.02	(1.36-2.99)	4.64E-04
HLA-DQB1*0402	0.185	0.114	0.85	2.02	(1.36-2.99)	4.64E-04
HLA-DQB1*05	0.154	0.214	0.91	0.64	(0.45-0.91)	0.014
HLA-DQB1*0501	0.076	0.153	0.93	0.43	(0.27-0.68)	2.95E-04
HLA-DQB1*0502	0.036	0.027	0.96	1.30	(0.63-2.7)	0.482
HLA-DQB1*0503	0.042	0.032	1.02	1.31	(0.68-2.55)	0.420
HLA-DQB1*06	0.240	0.239	1.01	1.03	(0.77-1.39)	0.842
HLA-DQB1*0602	0.052	0.083	1.03	0.65	(0.38-1.11)	0.119
HLA-DQB1*0603	0.148	0.095	0.95	1.73	(1.16-2.58)	7.25E-03
HLA-DQB1*0604	0.034	0.050	0.95	0.66	(0.33-1.29)	0.219
HLA-DPA1*01	0.839	0.846	0.96	0.93	(0.65-1.32)	0.667
HLA-DPA1*0103	0.831	0.844	0.95	0.88	(0.62-1.25)	0.485
HLA-DPA1*02	0.162	0.155	0.96	1.09	(0.76-1.55)	0.650
HLA-DPA1*0201	0.141	0.126	0.93	1.17	(0.8-1.72)	0.419
HLA-DPA1*0202	0.021	0.029	1.19	0.80	(0.37-1.72)	0.560
HLA-DPB1*01	0.034	0.033	1.08	1.01	(0.52-2)	0.968
HLA-DPB1*0101	0.034	0.033	1.08	1.01	(0.52-2)	0.968
HLA-DPB1*02	0.310	0.202	0.90	1.93	(1.41-2.66)	4,74E-05
HLA-DPB1*0201	0.310	0.196	0.90	1.99	(1.45-2.75)	2.56E-05
HLA-DPB1*03	0.115	0.100	0.86	1.10	(0.7-1.73)	0.678
HLA-DPB1*0301	0.115	0.100	0.86	1.10	(0.7-1.73)	0.678
HLA-DPB1*04	0.409	0.530	0.85	0.54	(0.41-0.73)	3.82E-05
HLA-DPB1*0401	0.320	0.415	0.92	0.62	(0.46-0.83)	1.14E-03
HLA-DPB1*0402	0.089	0.115	0.91	0.73	(0.46-1.15)	0.170
HLA-DPB1*10	0.018	0.023	0.94	0.72	(0.27-1.91)	0.511
HLA-DPB1*1001	0.018	0.023	0.94	0.72	(0.27-1.91)	0.511
HLA-DPB1*11	0.021	0.011	0.97	2.13	(0.75-6.08)	0.156
HLA-DPB1*1101	0.021	0.011	0.97	2.13	(0.75-6.07)	0.156
HLA-DPB1*13	0.018	0.015	0.96	1.23	(0.45-3.37)	0.690
HLA-DPB1*1301	0.018	0.015	0.96	1.23	(0.45-3.37)	0.690
HLA-DPB1*14	0.026	0.017	0.76	1.58	(0.55-4.56)	0.400
HLA-DPB1*1401	0.026	0.017	0.76	1.58	(0.55-4.57)	0.399
HLA-DPB1*15	0.013	0.002	0.98	9.59	(1.07-86.03)	0.043
HLA-DPB1*1501	0.013	0.002	0.98	9.59	(1.07-86.03)	0.043
HLA-DPB1*19	0.013	0.009	1.17	1.33	(0.44-4.06)	0.613
HLA-DPB1*1901	0.013	0.009	1.17	1.33	(0.44-4.06)	0.613

Supplementary Table 3. Association results for position 11 and 13 of *HLA-DRB1*. We performed a genome-wide association study in all JIA cases and unaffected controls (HC), in JIA with uveitis cases (JIA-U) and unaffected controls, and in JIA without uveitis (JIA non-U) and unaffected controls. We report here the association results in our dataset for Serine (Ser) at position 11 and previously-described Glycine (Gly) and Serine at position 13.

	Position	Amino acid residue	OR [95% CI]	P-value
JIA non-U vs HC	11	Ser	1.42 [0.50-4.00]	0.023
	13	Gly	4.16 [3.34-5.20]	1.69×10^{-6}
	13	Ser	0.91 [0.40-2.04]	0.53
JIA-U vs HC	11	Ser	3.46 [1.04-11.54]	1.41×10^{-8}
	13	Gly	6.03 [4.66-7.81]	3.54×10^{-8}
	13	Ser	1.61 [0.62-4.13]	0.018
All JIA vs HC	11	Ser	1.77 [0.56-5.66]	4.97×10^{-5}
	13	Gly	4.53 [3.43-5.97]	5.25×10^{-8}
	13	Ser	1.10 [0.46-2.66]	0.52

Supplementary Table 4. One-hundred and fourty-seven proteins selected from iris tissue proteome data for peptide binding affinity prediction to common *HLA-DRβ1* molecules. See **Materials and Methods** in the main article and **Supplementary Methods** for methodological details. Mass spectrometric proteome data from human iris tissues (2,959 nonredundant proteins) was used as a representative source of proteins present in iris tissue. Protein accession numbers were extracted to filter in UniProt (*Universal Protein Resource*) for proteins with a subcellular location annotation limited to the nucleus. 147 proteins fulfilled these criteria and their full length amino acid sequences were fed into the neural network of the netMHCIIpan3.1 server.

Entry	Entry name	Gene names	Subcellular location (Uniprot)
A8MWD9	RUXGL_HUMAN	*SNRPGP15*	Nucleus
O00442	RTCA_HUMAN	*RTCA/RPC/RPC1/RTC1/RTCD1*	Nucleus, nucleoplasm.
O43143	DHX15_HUMAN	*DHX15/DBP1/DDX15*	Nucleus. Nucleus, nucleolus.
O43172	PRP4_HUMAN	*PRPF4/PRP4*	Nucleus speckle
O43684	BUB3_HUMAN	*BUB3*	Nucleus. Chromosome, centromere, kinetochore
O43809	CPSF5_HUMAN	*NUDT21/CFIM25/CPSF25/CPSF5*	Nucleus
O60814	H2B1K_HUMAN	*HIST1H2BK/H2BFT/HIRIP1*	Nucleus. Chromosome.
O75367	H2AY_HUMAN	*H2AFY/MACROH2A1*	Nucleus
O75592	MYCB2_HUMAN	*MYCBP2/KIAA0916/PAM*	Nucleus
O75643	U520_HUMAN	*SNRNP200/ASCC3L1/HELIC2/KIAA0788*	Nucleus
O75934	SPF27_HUMAN	*BCAS2/DAM1*	Nucleus, nucleolus
O94901	SUN1_HUMAN	*SUN1/KIAA0810/UNC84A*	Nucleus inner membrane
P02545	LMNA_HUMAN	*LMNA/LMN1*	Nucleus. Nucleus envelope. Nucleus lamina.
P04908	H2A1B_HUMAN	*HIST1H2AB/H2AFM;/HIST1H2AE/H2AFA*	Nucleus. Chromosome.
P05455	LA_HUMAN	*SSB*	Nucleus
P06454	PTMA_HUMAN	*PTMA/TMSA*	Nucleus.
P08651	NFIC_HUMAN	*NFIC/NFI*	Nucleus.
P09012	SNRPA_HUMAN	*SNRPA*	Nucleus.
P09661	RU2A_HUMAN	*SNRPA1*	Nucleus.
P09874	PARP1_HUMAN	*PARP1/ADPRT/PPOL*	Nucleus. Nucleus, nucleolus.
P0C0S5	H2AZ_HUMAN	*H2AFZ/H2AZ*	Nucleus. Chromosome.
P10412	H14_HUMAN	*HIST1H1E/H1F4*	Nucleus. Chromosome.
P12004	PCNA_HUMAN	*PCNA*	Nucleus
P12956	XRCC6_HUMAN	*XRCC6/G22P1*	Nucleus
P13010	XRCC5_HUMAN	*XRCC5/G22P2*	Nucleus
P16403	H12_HUMAN	*HIST1H1C/H1F2*	Nucleus. Chromosome.
P17480	UBF1_HUMAN	*UBTF/UBF/UBF1*	Nucleus, nucleolus
P17844	DDX5_HUMAN	*DDX5/G17P1/HELR/HLR1*	Nucleus, nucleolus
P19387	RPB3_HUMAN	*POLR2C/A-152E5.7*	Nucleus
P19388	RPAB1_HUMAN	*POLR2E*	Nucleus
P20700	LMNB1_HUMAN	*LMNB1/LMN2/LMNB*	Nucleus inner membrane
P21291	CSRP1_HUMAN	*CSRP1/CSRP/CYRP*	Nucleus
P26368	U2AF2_HUMAN	*U2AF2/U2AF65*	Nucleus.
P26599	PTBP1_HUMAN	*PTBP1/PTB*	Nucleus.
P27694	RFA1_HUMAN	*RPA1/REPA1/RPA70*	Nucleus
P31942	HNRH3_HUMAN	*HNRNPH3/HNRPH3*	Nucleus
P35244	RFA3_HUMAN	*RPA3/REPA3/RPA14*	Nucleus.
P35637	FUS_HUMAN	*FUS/TLS*	Nucleus
P35659	DEK_HUMAN	*DEK*	Nucleus
P39019	RS19_HUMAN	*RPS19*	Nucleus
P39880	CUX1_HUMAN	*CUX1/CUTL1*	Nucleus.
P42166	LAP2A_HUMAN	*TMPO/LAP2*	Nucleus. Chromosome.
P43243	MATR3_HUMAN	*MATR3/KIAA0723*	Nucleus matrix.
P46063	RECQ1_HUMAN	*RECQL/RECQ1/RECQL1*	Nucleus

P50148	GNAQ_HUMAN	*GNAQ/GAQ*	Nucleus
P50402	EMD_HUMAN	*EMD/EDMD/STA*	Nucleus inner membrane
P51608	MECP2_HUMAN	*MECP2*	Nucleus.
P51991	ROA3_HUMAN	*HNRNPA3/HNRPA3*	Nucleus
P52272	HNRPM_HUMAN	*HNRNPM/HNRPM/NAGR1*	Nucleus, nucleolus
P52434	RPAB3_HUMAN	*POLR2H*	Nucleus, nucleolus
P52597	HNRPF_HUMAN	*HNRNPF/HNRPF*	Nucleus, nucleoplasm.
P52657	T2AG_HUMAN	*GTF2A2/TF2A2*	Nucleus.
P53999	TCP4_HUMAN	*SUB1/PC4/RPO2TC1*	Nucleus.
P54725	RD23A_HUMAN	*RAD23A*	Nucleus.
P55769	NH2L1_HUMAN	*SNU13/NHP2L1*	Nucleus, nucleolus
P61964	WDR5_HUMAN	*WDR5/BIG3*	Nucleus
P62805	H4_HUMAN	*HIST1H4A/*	Nucleus. Chromosome.
P62913	RL11_HUMAN	*RPL11*	Nucleus, nucleolus
P62995	TRA2B_HUMAN	*TRA2B/SFRS10*	Nucleus
P68400	CSK21_HUMAN	*CSNK2A1/CK2A1*	Nucleus
P78527	PRKDC_HUMAN	*PRKDC/HYRC/HYRC1*	Nucleus
P84243	H33_HUMAN	*H3F3A/H3.3A/H3F3/PP781;/H3F3B/H3.3B*	Nucleus. Chromosome.
Q00577	PURA_HUMAN	*PURA/PUR1*	Nucleus.
Q00688	FKBP3_HUMAN	*FKBP3/FKBP25*	Nucleus.
Q01081	U2AF1_HUMAN	*U2AF1/U2AF35/U2AFBP/FP793*	Nucleus
Q01130	SRSF2_HUMAN	*SRSF2/SFRS2*	Nucleus
Q03252	LMNB2_HUMAN	*LMNB2/LMN2*	Nucleus inner membrane
Q05639	EF1A2_HUMAN	*EEF1A2/EEF1AL/STN*	Nucleus
Q05925	HME1_HUMAN	*EN1*	Nucleus.
Q08170	SRSF4_HUMAN	*SRSF4/SFRS4/SRP75*	Nucleus speckle
Q09028	RBBP4_HUMAN	*RBBP4/RBAP48*	Nucleus.
Q09666	AHNK_HUMAN	*AHNAK/PM227*	Nucleus.
Q10570	CPSF1_HUMAN	*CPSF1/CPSF160*	Nucleus, nucleoplasm.
Q12874	SF3A3_HUMAN	*SF3A3/SAP61*	Nucleus speckle.
Q13151	ROA0_HUMAN	*HNRNPA0/HNRPA0*	Nucleus
Q13185	CBX3_HUMAN	*CBX3*	Nucleus
Q13243	SRSF5_HUMAN	*SRSF5/HRS/SFRS5/SRP40*	Nucleus.
Q13404	UB2V1_HUMAN	*UBE2V1/CROC1/UBE2V/UEV1/P/OKcl.19*	Nucleus
Q13435	SF3B2_HUMAN	*SF3B2/SAP145*	Nucleus
Q13547	HDAC1_HUMAN	*HDAC1/RPD3L1*	Nucleus
Q13595	TRA2A_HUMAN	*TRA2A*	Nucleus
Q13618	CUL3_HUMAN	*CUL3/KIAA0617*	Nucleus. Golgi apparatus.
Q13620	CUL4B_HUMAN	*CUL4B/KIAA0695*	Nucleus
Q14683	SMC1A_HUMAN	*SMC1A*	Nucleus
Q15029	U5S1_HUMAN	*EFTUD2/KIAA0031/SNRP116*	Nucleus
Q15369	ELOC_HUMAN	*ELOC/TCEB1*	Nucleus
Q15370	ELOB_HUMAN	*ELOB/TCEB2*	Nucleus
Q15393	SF3B3_HUMAN	*SF3B3/KIAA0017/SAP130*	Nucleus
Q15459	SF3A1_HUMAN	*SF3A1/SAP114*	Nucleus
Q15847	ADIRF_HUMAN	*ADIRF/AFRO/APM2/C10orf116*	Nucleus
Q16527	CSRP2_HUMAN	*CSRP2/LMO5/SMLIM*	Nucleus
Q16777	H2A2C_HUMAN	*HIST2H2AC/H2AFQ*	Nucleus. Chromosome.
Q1KMD3	HNRL2_HUMAN	*HNRNPUL2/HNRPUL2*	Nucleus
Q53GS9	SNUT2_HUMAN	*USP39/CGI-21/HSPC332/PRO2855*	Nucleus
Q5JTV8	TOIP1_HUMAN	*TOR1AIP1/LAP1*	Nucleus inner membrane
Q5SSJ5	HP1B3_HUMAN	*HP1BP3*	Nucleus
Q6P1N9	TATD1_HUMAN	*TATDN1/CDA11*	Nucleus
Q6P2Q9	PRP8_HUMAN	*PRPF8/PRPC8*	Nucleus speckle
Q6UXN9	WDR82_HUMAN	*WDR82/TMEM113/WDR82A*	Nucleus
Q6ZMZ3	SYNE3_HUMAN	*SYNE3/C14orf139/C14orf49/LINC00341*	Nucleus outer membrane
Q7Z6K1	THAP5_HUMAN	*THAP5*	Nucleus
Q86TJ2	TAD2B_HUMAN	*TADA2B/ADA2B*	Nucleus
Q8IUE6	H2A2B_HUMAN	*HIST2H2AB*	Nucleus. Chromosome.

Q8IXM6	NRM_HUMAN	*NRM/NRM29/UNQ555/PRO1112*	Nucleus inner membrane
Q8IYB8	SUV3_HUMAN	*SUPV3L1/SUV3*	Nucleus Mitochondrion matrix, mitochondrion nucleoid.
Q8N1F7	NUP93_HUMAN	*NUP93/KIAA0095*	Nucleus membrane
Q8NC56	LEMD2_HUMAN	*LEMD2*	Nucleus inner membrane
Q8NFW8	NEUA_HUMAN	*CMAS*	Nucleus
Q8TAQ2	SMRC2_HUMAN	*SMARCC2/BAF170*	Nucleus.
Q8TED0	UTP15_HUMAN	*UTP15*	Nucleus, nucleolus
Q8TEM1	PO210_HUMAN	*NUP210/KIAA0906/PSEC0245*	Nucleus, nuclear pore complex
Q92522	H1X_HUMAN	*H1FX*	Nucleus. Chromosome.
Q92841	DDX17_HUMAN	*DDX17*	Nucleus
Q92878	RAD50_HUMAN	*RAD50*	Nucleus
Q93084	AT2A3_HUMAN	*ATP2A3*	Nucleus membrane
Q96A72	MGN2_HUMAN	*MAGOHB/MAGOH2*	Nucleus
Q96AE4	FUBP1_HUMAN	*FUBP1*	Nucleus
Q96DI7	SNR40_HUMAN	*SNRNP40/PRP8BP/SFP38/WDR57*	Nucleus
Q96QR8	PURB_HUMAN	*PURB*	Nucleus
Q99733	NP1L4_HUMAN	*NAP1L4/NAP2*	Nucleus.
Q99959	PKP2_HUMAN	*PKP2*	Nucleus
Q9BQ69	MACD1_HUMAN	*MACROD1/LRP16*	Nucleus
Q9BU61	NDUF3_HUMAN	*NDUFAF3/C3orf60*	Nucleus
Q9BUJ2	HNRL1_HUMAN	*HNRNPUL1/E1BAP5/HNRPUL1*	Nucleus
Q9BVC6	TM109_HUMAN	*TMEM109*	Nucleus outer membrane
Q9BXJ8	T120A_HUMAN	*TMEM120A/TMPIT*	Nucleus inner membrane
Q9H0W9	CK054_HUMAN	*C11orf54/LP4947/PTD012*	Nucleus
Q9H1Z4	WDR13_HUMAN	*WDR13*	Nucleus
Q9HB07	MYG1_HUMAN	*C12orf10*	Nucleus
Q9NQG5	RPR1B_HUMAN	*RPRD1B/C20orf77/CREPT*	Nucleus
Q9NRG9	AAAS_HUMAN	*AAAS/ADRACALA/GL003*	Nucleus, nuclear pore complex
Q9NY12	GAR1_HUMAN	*GAR1/NOLA1*	Nucleus, nucleolus. Cajal body.
Q9NZQ3	SPN90_HUMAN	*NCKIPSD/AF3P21/SPIN90*	Nucleus.
Q9P0M6	H2AW_HUMAN	*H2AFY2/MACROH2A2*	Nucleus
Q9P2K5	MYEF2_HUMAN	*MYEF2/KIAA1341*	Nucleus.
Q9UH99	SUN2_HUMAN	*SUN2/FRIGG/KIAA0668/RAB5IP/UNC84B*	Nucleus inner membrane
Q9UHX1	PUF60_HUMAN	*PUF60/FIR/ROBPI/SIAHBP1*	Nucleus
Q9UJC5	SH3L2_HUMAN	*SH3BGRL2/FASH3*	Nucleus
Q9UKK9	NUDT5_HUMAN	*NUDT5/NUDIX5/HSPC115*	Nucleus
Q9UKM9	RALY_HUMAN	*RALY/HNRPCL2/P542*	Nucleus
Q9UQE7	SMC3_HUMAN	*SMC3/BAM/BMH/CSPG6/SMC3L1*	Nucleus. Chromosome, centromere.
Q9Y2S7	PDIP2_HUMAN	*POLDIP2/PDIP38/POLD4/HSPC017*	Nucleus
Q9Y2X3	NOP58_HUMAN	*NOP58/NOL5/NOP5/HSPC120*	Nucleus, nucleolus. Nucleoplasm
Q9Y333	LSM2_HUMAN	*LSM2/C6orf28/G7B*	Nucleus
Q9Y3B4	SF3B6_HUMAN	*SF3B6/SAP14*	Nucleus
Q9Y3E1	HDGR3_HUMAN	*HDGFRP3/HDGF2/CGI-142*	Nucleus
Q9Y3Z3	SAMH1_HUMAN	*SAMHD1/MOP5*	Nucleus

Supplementary Table 5. Binding affinity changes in various DRβ1 configurations. The average binding affinities for peptide data set based on human iris proteome (see methods) of 6 *HLA-DRB1* alleles (*03:01, *08:01, *11:01, *12:01, *13:01, and *14:01) that contain serine-11 (ser11+) and 7 *HLA-DRB1* alleles (*01:01, *04:01, *07:01, *09:01, *10:01, *15:01, *16:01) that have other amino acids at this position (Ser11-). We computed the ratio of the average binding affinity for Ser11+ over Ser11- for the entire set of peptides, potential ligands for Ser11+ with a binding affinity stronger than (half maximal inhibitory concentration IC_{50}) 500 nM, and peptides with high binding affinity for Ser11 (IC_{50}<50nM).

Peptide Dataset		Peptides (n)	Median	*P value*
	Ser11-	83686	0.312	
	Ser11+	83686	0.323	3.4×10^{-136} [a]
1	Ratio (Ser11+/Ser11-)	83686	1.007	1.5×10^{-150} [b]
2	Ratio (Ser11+<500nM/ Ser11-)	21598	1.096	5.4×10^{-109} [c]
3	Ratio (Ser11+<50nM/Ser11-)	1295	1.153	1.6×10^{-31} [d]

a. Wilcoxon matched pairs test, Ser11+ vs Ser11-
b. One-sample Wilcoxon Test, H0 = M = 1.000
c. Wilcoxon matched pairs test 1 vs 2
d. Wilcoxon matched pairs test 2 vs 3

Supplementary Table 6. Association results for known, associated variants in uveitis and JIA. We performed a genome-wide association study in (1) all JIA cases and unaffected controls and (2) uveitis and unaffected controls. We report here the association results in our dataset for previously-described associations in both JIA and uveitis. Originally reported odds ratios and p-values are also provided.

Sources for previously known associations (Pub, publications, column 1) are:
(1) Hinks, A. *et al.* Ann Rheum Dis. 2016 Apr;76(4):765-772.[1]
(2) Zulian, F. *et al.* J Rheumatol. 2002 Nov;29(11):2446-53.[2]
(3) Zeggini, E. *et al.* Rheumatology (Oxford). 2006 Aug;45(8):972-4.[3]
(4) Yanagimachi, M. *et al.* J Hum Genet. 2011 Mar;56(3):196-9.[4]
(5) Angeles-Han, ST *et al.* Invest Ophthalmol Vis Sci. 2015 Sep;56(10):6043-8.[5]

Pub	Phenotype	Amino acid or HLA allele	Previously -described associations		Current Study		
			OR	***P-value***	***AF***	***OR [95% CI]***	***P-value***
(1) JIA (n=522) vs. unaffected (Non-JIA) controls (n=398)							
1	RF-negative polyarthritis	*HLA-DRB1* pos. 13 glycine	2.02	4.29×10^{-99}	0.121	5.05 [3.29 - 7.75]	1.13×10^{-13a}
1	JIA	*HLA-DRB1* pos. 13 histidine	2.44	3.65×10^{-31}	0.104	0.37 [0.25 - 0.55]	1.44×10^{-6}
(2) JIA-associated uveitis (n=192) vs. unaffected (Non-JIA) controls (n=398)							
2	Uveitis	*HLA-A*19*	2.87	NR	*Absent from the SNP2HLA imputation reference panel*		
2	Uveitis	*HLA-B*22*	4.51	NR			
3	Uveitis	*HLA-DRB1*13*	3.4	2.0×10^{-3}	0.156	1.79 [1.17 - 2.72]	6.72×10^{-3}
4	Uveitis	*HLA-A*0206*	11.7	$<1.0 \times 10^{-3}$	Absent from the SNP2HLA imputation reference panel		
5	Uveitis	*HLA-DRB1*11*	2.2	0.023	0.128	3.36 [2.10 - 5.49]	1.26×10^{-6}
5	Uveitis	*HLA-DRB1*08*	9.6	<0.0001	0.079	12.49 [6.73 - 23.19]	1.29×10^{-15a}
5	Uveitis	*HLA-DRB1*13*	2.3	0.01	0.16	1.48 [1.02 - 2.15]	0.04

OR, odds ratio. NR, not reported. AF, allele (or amino acid) frequency. For the case of amino acids, the frequency shown is the frequency of the amino acid being present (as opposed to absent) in a sample.
[a] excluding the covariate for study phase; results are NA when correcting for study phase

Supplementary Table 7. Summary of data quality control. Before performing imputation, we ran sample-level and SNP-level data quality control, following standard genome-wide association quality control steps. All steps, thresholds, and number of removed samples and SNPs are summarized here. All quality control was performed using Plink 1.9 and principal component analysis was performed using EIGENSTRAT (see **Supplementary Methods**).

QC Step	Phase 1		Phase 2	
	Exclusion Threshold	Failures	Exclusion Threshold	Failures
Sample QC				
Sample missingness	> 5%	6	Identical to Phase 1	8
Phenotypic-genotypic sex concordance	Males: X chr. inbreeding coeff < 0.8 Females, X chr. inbreeding coeff > 0.2 X chr inbreeding coeff > 0.2 and < 0.8	4	Identical to Phase 1	3
PCA	+/- 6 SD from means of PC1 and PC2 from the HapMap 3 European-ancestry populations	16	Identical to Phase 1	19
Inbreeding	+/- 3 SD from distribution mean	0	Identical to Phase 1	3
Relatedness	Pi-hat > 0.125	7	Identical to Phase 1	1
Total unique failures	--	31		27
SNP QC				
Frequency	< 1%	77,329	Identical to Phase 1	78,476
HWE	$p < 1x\ 10^{-6}$ in controls	575	$p < 1 \times 10^{-3}$ in controls	3
Differential missingness	p < 0.05	65,065	$p < 1 \times 10^{-3}$	2,663
Missingness	> 5%	7,112	Identical to Phase 1	15,074
Total unique failures	--	138,792	--	93,627

PCA, principal component analysis; SD, standard deviation; HWE, Hardy-Weinberg equilibrium. The European-ancestry populations included in HapMap 3 are the CEU (Northern and Western European-ancestry individuals living in Utah) and the TSI (Toscans in Italy).

SUPPLEMENTARY METHODS

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)).[6,7] All patients were screened by an ophthalmologist according to the guidelines of the Academy of Pediatrics and patients with no clinical signs of uveitis had an ophthalmologic follow-up of at least 4 years after onset of JIA patients with at least 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.[8]

The majority (92%) of included JIA cases fall into the following *International League of Associations for Rheumatology* (ILAR) categories: (i) oligoarticular extended, (ii) oligoarticular persistent, and (iii) RF-negative polyarticular JIA (**Table 1** in main article). These common categories of JIA are also particularly prone to uveitis and have recently been shown to be genetically highly similar in HLA associations.[1]

DNA material of JIA patients with and without uveitis from Phase 1 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 based in the Netherlands). Samples from Phase 2 were collected within 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.[9]

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.

Sample collection and genotyping

In Phase 1, we genotyped 137 JIA-uveitis cases, 247 non-uveitis JIA samples, and 398 population-level controls without JIA or uveitis. Samples and SNPs underwent quality control (QC) (please see below) to remove those samples and SNPs with low-quality genotyping data. After QC, 126 JIA-uveitis cases, 231 non-uveitis JIA samples, and 394 population-level controls remained (**Table 1** in main article).

In Phase 2, an additional 77 JIA-uveitis cases and 115 JIA without uveitis samples (total N = 192) were genotyped on the same array. Samples and SNPs underwent QC nearly identical to that of Phase 1. After QC, 66 JIA-uveitis cases and 99 non-uveitis JIA samples remained for analysis.

Data quality control

Genotyping and quality control

All samples were genotyped using the Infinium HumanOmniExpress-24v1.1 and v1.2 arrays, which contain ~700,000 SNPs. For Phase 1, all JIA cases (with and without uveitis) were genotyped together; unaffected controls were genotyped on the same array, but as a separate batch. All Phase 2 samples were genotyped together.

Sample-level quality control

Before performing imputation, we ran sample-level and SNP-level data quality control, following standard genome-wide association quality control steps. All steps, thresholds, and number of removed samples and SNPs are summarized in **Supplementary Table 7**. Briefly, we used PLINK 1.9 to first check sample-level missingness across all samples and removed all samples with missingness >5%.[10] Next, for the remainder of sample-level quality control, we reduced the dataset to only high-quality SNPs: SNPs with minor allele frequency (MAF) > 10%; SNPs outside the MHC (chromosome 6), lactase locus (chromosome 2), and the inversions on chromosomes 8 and 17; SNPs with missingness < 0.1%; and SNPs linkage disequilibrium (LD) pruned at an LD (r^2) threshold of 0.2.

With this high-quality set of SNPs, we performed principal component analysis (PCA) using EIGENSTRAT.[11] We defined samples to be of European ancestry if their principal component (PC) 1 and PC 2 values were within 6 standard deviations of the European-ancestry populations included in the HapMap 3 dataset (the CEU population, samples of Northern and Western European ancestry living in Utah; and the TSI population, Toscans living in Italy). In addition, we calculated inbreeding coefficients for all samples and removed samples further than 3 standard deviations from the distribution. Related samples (pi-hat > 0.125) were also removed. Finally, using all SNPs available on chromosome X, we performed a sex check to identify samples with mismatching genotypic and phenotypic sex; samples with mismatching genotype and phenotype information were removed from the analysis.

SNP-level quality control

We dropped failing samples from the data and then proceeded to perform SNP QC. All SNPs with missingness >5% were removed, as were SNPs with frequency <1% (too rare to be analyzed in the logistic regression framework, given the sample size). SNPs out of Hardy-Weinberg equilibrium in controls or across the full dataset were removed ($p < 1 \times 10^{-6}$ in Phase 1; $p < 1 \times 10^{-3}$ in Phase 2), as were SNPs with significant differential missingness between cases and controls ($p < 5 \times 10^{-2}$ in Phase 1; $p < 1 \times 10^{-3}$ in Phase 2).

Once sample- and SNP-level QC were complete and the failing samples and SNPs had been removed from the data, we performed a second iteration of PCA to ensure that cases and controls were overlapping in PCA space (i.e., there was no obvious population stratification in the data) (**Supplementary Figure 10**).

Prephasing and imputation

Prephasing

Once sample- and SNP-level were complete, the Phase 1 and Phase 2 data were phased separately, as they were genotyped and QC'd separately, using SHAPEIT2.[12] As the sample size was >100 samples, prephasing was run without a reference panel, per the SHAPEIT2 recommendations.[12]

Genome-wide imputation

Following the prephasing, we imputed all samples using the imputation reference panel constructed through whole-genome sequencing of 2,504 samples in 1000 Genomes Project (Phase 3).[13] Briefly, the 1000 Genomes Project Phase 3 data is comprised of samples collected from the Americas, Africa, East Asia, Europe and South Asia. Samples were whole-genome sequenced at ~80x coverage across the exome and at ~4x coverage in non-exome regions. We imputed the prephased haplotypes using IMPUTE2; the data were phased in windows 5 megabases long, with a buffer region of 250kb.[14,15] We set the effective sample size (Ne) to 20,000, per IMPUTE2 recommendations, and set k_hap to 1,000, as our sample was of European ancestry and the 1000 Genomes Phase 3 imputation reference panel contains ~1,000 haplotypes from European-ancestry individuals.[13]

Imputation of HLA classical alleles and amino acids

To impute amino acids and HLA alleles in the MHC, we used the SNP2HLA pipeline.[16] In short, the SNP2HLA pipeline uses a reference panel assembled through HLA typing of 5,225 European-ancestry individuals collected by the Type 1 Diabetes Genetics Consortium.[17] The panel includes SNPs and amino acids in the MHC, as well as HLA types from the Class I (*HLA-A*, *HLA-B*, and *HLA-C*) and Class II (*HLA-DRB1*, *HLA-DQA1*, *HLA-DQB1*, *HLA-DPA1*, and *HLA-DPB1*) HLA genes. HLA types are imputed to 2- and 4-digit resolution. The SNP2HLA pipeline uses BEAGLE to phase the data and then impute.[18]

Post-imputation, we checked the sum of the dosages across each HLA gene for each individual. Samples with dosages >2.5 at any one of the HLA genes (induced through imprecision in the imputation) were dropped from further analysis.

Association testing

Genome-wide association testing

To perform genome-wide association testing, we coded JIA samples with uveitis as cases and JIA samples without uveitis as controls. For genome-wide association testing in the Phase 1 and Phase 2 data, we kept those SNPs with a minor allele frequency (MAF) > 1% and with an imputation quality score > 0.7; >9.2M variants were available in both the Phase 1 and Phase 2 after imputation and filtering (82.6% and 77.6% of SNPs in Phase 1 and Phase 2 respectively had an info score ≥ 0.9; **Supplementary Table 1**). For MHC association testing, we analyzed only those variants with MAF > 1%. No imputation quality filter was applied to the MHC-specific imputed data; 97.7% and 96.4% of MHC variants in Phase 1 and Phase 2, respectively, had an imputation quality score > 0.9 (**Supplementary Table 1**).

First, we performed a genome-wide association study (GWAS) in Phase 1 and Phase 2 individually, to check the overall behavior of the genome-wide test statistics ($lambda_{Phase1}$ = 1.008, $lambda_{Phase2}$ = 1.017). GWAS were performed using PLINK 1.9 using an additive logistic regression model, correcting for the top two principal components and sex.[10,19] Data were then meta-analyzed using METAL.[20] To ensure we were analyzing SNPs with high-quality imputation, we only analyzed common SNPs (MAF > 1%) with imputation quality (info) score > 0.7. In the Phase 1 data, additional GWAS were performed comparing samples with JIA and uveitis to unaffected controls, as well as JIA samples without uveitis to unaffected controls; results were concordant with studies previously performed in these phenotypes (**Supplementary Table 6**).

Association testing in the MHC

Similar to the genome-wide association testing, additive logistic regression in the Phase 1 and Phase 2 data was first performed separately to check the overall behavior of the data. Then, the dosages were merged together, and logistic regression was performed on the dosage data using PLINK 1.9, correcting for the top 5 principal components, sex, and phase.[10] 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). To identify potential independent signals within the MHC, we performed a follow-up regression by conditioning on the top-most variant (**Figure 1** in main article).

In silico peptide binding prediction to *HLA-DRβ1*

Proteome data from human iris tissues (2,959 nonredundant proteins) was used as a representative source of proteins present in iris tissue.[21] JIA-uveitis patients commonly have ANAs and antibodies directed to iris tissues, thus, we focused on nuclear iris proteins to generate a potentially disease-relevant dataset. Protein accession numbers were extracted to filter in UniProt (Universal Protein Resource) for proteins with a subcellular location annotation limited to the nucleus.[22] 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. The netMHCIIpan algorithm was selected for its high quality performance in peptide binding prediction for any MHC class II allele for which the sequence is identified, including those for whom only few experimental peptide binding affinities (limited allele-specific training data) are available.[23] We ascertained this by testing the predicted binding affinity of several hundreds of experimentally identified 15-mer peptide ligands eluted from HLA-DRβ1:0, which revealed that >95% were correctly picked up by netMHCIIpan as ligands for DRβ1:01 (IC_{50} <500nM, data not shown).[24]

Next, we tested the predicted affinities of all 83,686 overlapping 15-mer peptides from the selected 147 proteins in *netMHCIIpan3.1*. that tentatively could be presented by HLA-DR for binding to representative four-digit alleles of *HLA-DRB1* (**01:01, *03:01, *04:01, *07:01, *08:01, *09:01, *10:01, *11:01, *12:01, *13:01, *14:01, *15:01,* and **16:01*). These alleles account for 79% of *HLA-DRβ1* alleles in the JIA cases. 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 (alleles) with similar predicted binding preferences, we performed unsupervised hierarchical clustering. Heatmaps were created based on the Euclidean distance measure and the Ward's linkage method using the MetaboAnalyst server.[25] We computed the ratio of the average binding affinity of HLA-DRβ1 molecules that contain Serine-11 in the peptide-binding groove (**03:01, *08:01, *11:01, *12:01, *13:01,* and **14:01*) over the average binding affinity of HLA-DRβ1 proteins that have other amino acids at this position (**01:01, *04:01, *07:01, *09:01, *10:01, *15:01,* and **16:01*) as a measure for the overall difference in predicted binding affinity for each peptide. A frequency distribution of the ratio of the binding affinities was plotted for the entire set of peptides (including low and nonbinding peptides), potential ligands for DRβ1 with a binding affinity stronger than half maximal inhibitory concentration (IC_{50}) of 500 nM, or peptides with high binding affinity for Serine-11 encoding alleles (IC_{50}<50nM).

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Chapter 4

Ocular fluid analysis in children reveals interleukin-29/interferon-λ1 as a biomarker for juvenile idiopathic arthritis-associated uveitis

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ABSTRACT

Objective
Childhood uveitis is a visual-threatening inflammatory eye disease commonly attributed to juvenile idiopathic arthritis (JIA). The pathogenesis is poorly understood, which makes clinical management challenging. We analyzed soluble mediators in ocular fluid (aqueous humor [AqH]) and serum from children with JIA-associated uveitis and common childhood uveitis to identify potential biomarkers and investigate the ocular microenvironment of this sight-threatening eye disease.

Methods
AqH (n=73) and paired serum (n=66) samples were analyzed for 51 soluble inflammatory mediators by multiplex immunoassay. Twenty-one children with JIA-associated uveitis were compared with 15 with chronic anterior uveitis without arthritis, 29 children with noninfectious idiopathic uveitis, and 8 children with noninflammatory conditions (controls). For visualization of the joint effect of multiple mediators, we used the radial coordinate visualization (Radviz) method. Optimal biomarker level cutoffs were also determined.

Results
The levels of interleukin-29 (IL-29)/interferon-λ1 (IFNλ1) were decreased ($P<0.001$) and the levels of latency-associated peptide and osteoprotegerin were increased ($P=0.002$ and $P=0.001$, respectively) in samples of AqH, but not in serum, from patients with JIA-associated uveitis. Multivariate analysis correcting for disease activity and treatment revealed that intraocular levels of IL-29/IFNλ1 were specifically decreased in patients with JIA-associated uveitis as compared to those with idiopathic uveitis. Indeed, JIA-associated uveitis patients and idiopathic uveitis patients showed distinct profiles of intraocular soluble mediatos. IL-29/IFNλ1 showed a high area under the curve value (0.954), with 23.5 pg/ml as the optimal cutoff value.

Conclusion
We identified IL-29/IFNλ1 as an intraocular biomarker for JIA-associated uveitis, which suggests that aberrant IFNλ signaling might be important in JIA-associated uveitis and distinct from other forms of childhood uveitis.

INTRODUCTION

Childhood uveitis is a sight-threatening inflammatory eye disease and constitutes 5-10% of all uveitis cases.[1] The vast majority of childhood uveitis is related to juvenile idiopathic arthritis (JIA). Noninfectious chronic anterior uveitis is the most common extraarticular complication of JIA and occurs in 10-45% of children with JIA, especially in children with the oligoarticular subtype.[2-5] In general, JIA precedes uveitis; however, ~20% of patients develop anterior uveitis before the onset of JIA.[6,7] Curiously, there are also children who have uveitis that is clinically identical to the chronic anterior uveitis associated with JIA, but who generally do not develop arthritis (referred to herein as chronic anterior uveitis).

The underlying mechanisms that link uveitis and JIA remain enigmatic.[8] Regardless, JIA-associated uveitis, like either JIA or noninfectious uveitis, is thought to be mediated by currently poorly understood multifactorial aberrant activation of innate and adaptive immune pathways, with the disturbed balance of T helper cell and T regulatory cell subsets being the most well-studied contributors.[8-12]

Analysis of the aqueous humor (AqH) has proven to be a useful tool for extracting information on crucial factors that orchestrate the ocular microenvironment in childhood uveitis.[13] Emerging innovative technological developments, such as multiplex bead-based immunoassays, have made it possible to simultaneously measure and quantify a large variety of soluble immune mediators in small sample volumes.[14] Biomarkers in AqH from patients with various childhood uveitis entities, including JIA-associated uveitis, are currently lacking. The main goal of the current study was to analyze immune mediators in AqH from a unique cohort of children with JIA-associated uveitis and related or common childhood uveitis to identify potential new soluble biomarkers and underlying immunological patterns of this vision-threatening eye disease in children.

PATIENTS AND METHODS

Study population and clinical sampling

This study was conducted in compliance with the principles of the Declaration of Helsinki and was approved by the local review board. After obtaining informed consent, from both of the parents if patients were <12 years of age or from the parents and the patient if the patients were ≥ 12 years of age, we collected samples of aqueous humor (AqH) from 73 children (73 eyes) who were younger than 18 years between 2003 and 2014 and attended the Department of Ophthalmology at the University Medical Center of Utrecht. Paired serum samples were available for 66 of these children. Samples were collected during surgery indicated for complications (such as glaucoma and cataract) or were the remainder of samples obtained for diagnostic purposes at the time of anterior chamber paracentesis for infectious causes. Samples were immediately stored at -80°C after collection.

A total of 73 children were studied. Of these, 21 had a diagnosis of JIA-associated uveitis, 15 had chronic anterior uveitis, 29 had noninfectious idiopathic intermediate uveitis or panuveitis with anterior chamber involvement (referred to herein as idiopathic uveitis), and 8 had noninflammatory conditions (AqH samples from 5 patients with cataract and 3 with glaucoma). The latter group served as controls. Uveitis was diagnosed according to the recommendations of the International Uveitis Study Group [15], as determined by an ophthalmologist with a specialty in childhood uveitis. Evaluation of systemic disease,

including JIA based on the International League of Associations for Rheumatology criteria[16], was done by pediatric rheumatologists or immunologists. All children with the diagnosis of JIA-associated uveitis were rheumatoid factor-negative and had polyarticular or oligoarticular JIA. Children who had ocular inflammation that clinically resembled JIA-associated uveitis, but had no detectable arthritis as determined by a pediatric rheumatologist, were diagnosed as having chronic anterior uveitis. Additional clinical information that was collected included the age at disease onset, sex, duration of disease, localization of disease, antinuclear antibody (ANA) status, disease activity, and treatment. Uveitis was considered active if at least 1+ cells (6-15 cells per slit beam according to the criteria of the Standardization of Uveitis Nomenclature Working Group[17]) were found in the anterior chamber and/or vitreous body upon ophthalmologic examination prior to sampling.

Multiplex immunoassay

A total of 73 AqH samples and 66 serum samples were analyzed using an in-house developed and validated multiplex immunoassay based on Luminex technology [18]. Fifty-one soluble mediators were analyzed in AqH and serum samples (**Supplementary Tables 1** and **2**, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.39621 /abstract). For statistical analysis, concentrations below the detection limit were converted to the lowest point on the calibration curve multiplied by 0.5. When >25% of the measurements were below the detection limit, the mediator was excluded from further analysis, unless >90% of the measurements were detected in 1 specific subgroup.

Thirty-five of 51 soluble mediators were detected in AqH samples (**Supplementary Table 1**). Interleukin-12p70 (IL-12p70), IL-23p19, thymic stromal lymphopoietin, angiopoeietin 1, thymus and activation-regulated chemokine, IL-13, IL-1 receptor antagonist (IL-1Ra), IL-1β, IL-2, IL-4, IL-17, tumor necrosis factor (TNF), interferon-γ (IFN-γ), leukocyte-associated immunoglobulin-like receptor 1, macrophage inflammatory protein 1α (MIP-1α), and IL-10 were below the detection range in >25% of the AqH samples and excluded from further analyses. Thirty-seven of 51 soluble mediators were detected in serum samples (**Supplementary Table 2**). IL-29/IFN λ1, IL-12p70, nerve growth factor, S100 A8 protein, thrombopoietin, IL-1Ra, IL-1β, IL-2, IL-4, IL-17, TNF, IFNγ, LAIR-1, and IL-10 were below the detection range in >25% of the serum samples and were excluded from further analyses.

Statistical Analyses

IBM SPSS Statistics v20 software and GraphPad Prism v6 software were used for statistical analyses. Since cytokine concentrations in the AqH samples were abnormally distributed, the nonparametric Kruskal-Wallis test with Dunn's post hoc test was used to compare the median of the cytokine levels between the different groups. Where appropriate, the Pearson chi-square test with Bonferroni correction for multiple testing was used to compare categorical data. Correlation was tested by Spearman's rho (for continuous variables) or with Cramér's V (for nominal variables). Multivariate logistic regression analysis was used to test for independency. *P* values less than 0.05 were considered statistically significant.

For visualization of the joint effect of soluble mediators in AqH, we used the radial coordinate visualization (Radviz) method to project high-dimensional data into the orthogonal space, which uses a k-nearest neighbor classifier to evaluate the validity of the projection (Orange Data Mining software).[19] The best projection is chosen via the VizRank method. Of 5,000 projections, the soluble mediators with the best capacity to distinguish

between the groups were plotted as described in the Results section. To test the ability of potential biomarkers to discriminate between patient groups, receiver operating characteristic (ROC) curve analysis was performed in MetaboAnalyst 3.0.[20]

RESULTS

General characteristics of the study population

The 4 patient groups had a comparable ratio of girls to boys (range 41-63% female per group) (**Table 1**). In the JIA-associated uveitis group, the median duration of JIA at the time of sampling was 6.4 years (range 1.0-14.0 years). In 16 patients with JIA-associated uveitis (76%), the onset of JIA occurred before the onset of uveitis, and uveitis was initially diagnosed in 5 patients (24%) in this group. The mean age of the patients was similar in all uveitis groups (mean ± SD range 8.8 ± 3.1 years to 10.9 ± 3.7 years), but it was significantly decreased in the control group (mean ± SD 4.9 ± 3.4 years; P=0.002). Seventy-two percent of the idiopathic uveitis patients had active uveitis, as compared to 5% of the JIA-associated uveitis patients (P<0.001).

Of the 21 JIA-associated uveitis patients, 13 (62%) had inactive arthritis, 4 (19%) had active arthritis, and in the other 4 patients (19%), the arthritis status at the time of sampling was unknown. All patients with JIA-associated uveitis, 31% with idiopathic uveitis, and 67% with chronic anterior uveitis were receiving systemic immunomodulatory therapy (IMT) (P<0.001) (**Table 1**). The duration of uveitis at the time of sampling was significantly greater in the JIA-associated uveitis patients (median 5.8 years [range 1.1-14.0 years]) than in the chronic anterior uveitis patients (median 1.4 years [range 0.1-7.8 years]; P=0.015) and in the idiopathic uveitis patients (median 0.6 years [range 0.0-9.0 years]; P<0.001). Accordingly, the uveitis activity, systemic IMT, and disease duration were all significantly correlated with each other (r^2=0.81 for the uveitis activity versus the uveitis duration, Cramér's V=0.56 for the uveitis activity versus systemic IMT, and r^2=0.74 for the uveitis durations versus systemic IMT; P<0.001 for each comparison).

Table 1. Characteristics of the study patients, by diagnostic group*

	Total	JIA-associated uveitis	Chronic anterior uveitis	Idiopathic uveitis	Control	*P*, across groups	*P*, JIA-associated uveitis versus†		
							Chronic anterior uveitis	Idiopathic uveitis	Control
No. of patients	73	21	15	29	8	-	-	-	-
No. (%) female	39 (53)	13 (62)	9 (60)	12 (41)	5 (63)	0.420‡	-	-	-
Age, mean ± SD years	9.7 (3.9)	10.6 (3.3)	8.8 (3.1)	10.9 (3.7)	4.9 (3.4)	**0.002**§	0.489	**1.000**	**0.006**
No. (%) with active uveitis	27 (37)	1 (5)	5 (33)	21 (72)	-	**<0.001**‡	**0.034**	**<0.001**	NA
Duration of uveitis, median (range) years	1.5 (0.0-14.0)	5.8 (1.1-14.0)	1.4 (0.1-7.8)	0.6 (0.0-9.0)	-	**<0.001**§	**0.015**	**<0.001**	NA
No. (%) receiving systemic treatment¶	40 (55)	21 (100)	10 (67)	9 (31)	0 (0)	**<0.001**‡	**0.012**	**<0.001**	**<0.001**
No. (%) with paired serum samples	66 (90)	20 (95)	14 (93)	25 (86)	7 (88)	0.710‡	-	-	-
Storage time, median (range) years	2.5 (0.1-11.0)	1.9 (0.3-9.5)	2.6 (0.1-11.0)	2.5 (0.4-6.2)	3.2 (0.3-4.5)	0.684§	-	-	-
No. with known ANA status	60	19	15	26	0		-	-	-
No. (%) ANA positive	36 (60)	17 (89)	10 (67)	9 (35)	0 (0)	**<0.001**‡	0.363	**0.003**	**<0.001**

*Age, number (%) with active uveitis, and number (%) receiving systemic treatment were determined at the time of aqueous humor sampling. Storage time was the period from the time of sampling to the time of analysis. The control group consisted of patients with noninflammatory conditions. ANA = antinuclear antibody.

† Determined by post hoc test with Bonferroni correction for multiple testing.

‡ Determined by Pearson's chi-square test.

§ Determined by Kurskal-Wallis test

¶ Systemic treatment consisted of either tumor necrosis factor inhibitor plus systemic corticosteroids and/or methotrexate (MTX) (11 patients: 8 with juvenile idiopathic arthritis [JIA]–associated uveitis, 1 with chronic anterior uveitis, 1 with idiopathic uveitis, and 1 control), MTX plus systemic corticosteroids (20 patients; 10 with JIA-associated uveitis, 8 with chronic anterior uveitis, and 2 with idiopathic uveitis), MTX only (2 patients; 1 with JIA-associated uveitis and 1 with idiopathic uveitis), systemic corticosteroids only (6 patients; 1 with JIA-associated uveitis, 1 with chronic anterior uveitis, and 4 with idiopathic uveitis), or mycophenolate mofetil plus systemic corticosteroids (2 patients; 1 with JIA-associated uveitis and 1 with idiopathic uveitis).

Analysis of aqueous humor for soluble mediators

Data for the 35 mediators detected in the AqH samples from the patients are shown in Supplementary **Table 1.** Sixteen mediators were not detected in sufficient quantity in the cohorts and were excluded from further analysis as described in Patients and Methods. Levels of IL-29/IFN-1 were significantly decreased in patients with JIA-associated uveitis (median 18.8 pg/ml [range 2.9-29.0]) as compared to the levels in the other 3 patient groups (median of 27.3 pg/ml [range 5.7-40.9] in the chronic anterior uveitis group, 32.7 pg/ml [range 6.9-75.2] in the idiopathic uveitis group, and 29.1 pg/ml [range 26.8-42.5] in the control group; $P<0.05$, $P<0.001$, and $P<0.001$, respectively) (**Figure 1**). Latency associated peptide (LAP) and osteoprotegerin (OPG) levels were significantly increased in the JIA-associated uveitis group as compared to the idiopathic uveitis and the control groups ($P=0.036$ and $P=0.003$, respectively, for LAP; $P=0.030$ and $P<0.001$, respectively, for OPG) (**Figure 1**).

Brain-derived neurotrophic factor (BDNF), IL-6, and S100A8 were significantly decreased in JIA-associated uveitis patients compared to idiopathic uveitis patients, but not compared to controls. The levels of 12 additional mediators were significantly different between JIA-associated uveitis patients and controls, but were similar between JIA-associated uveitis patients and idiopathic uveitis patients (**Supplementary Table 1**). Except for IL-29/IFNλ1, no significant differences between the JIA-associated uveitis and chronic anterior uveitis groups were found. Complement C5a levels were significantly increased in patients with chronic anterior uveitis (median 170.8 pg/ml) as compared to patients with idiopathic uveitis (median 44.8 pg/ml; $P=0.013$), and IL-22 levels were increased in chronic anterior uveitis patients (median 52.2 pg/ml) as compared to idiopathic uveitis patients (median 38.0 pg/ml; $P=0.004$).

Since most patients with JIA-associated uveitis had inactive uveitis and were receiving systemic IMT, which could influence the levels of immune mediators in the AqH, we next compared the levels of soluble mediators in patients with inactive uveitis and patients receiving systemic IMT in the different diagnostic groups. Only IL-29/IFNλ1 remained specifically and significantly decreased in patients with JIA-associated uveitis as compared with either patients with inactive uveitis or those receiving systemic IMT (**Figure 2**), with the exception of those with chronic anterior uveitis.

The levels of LAP, OPG, IL-6, S100A8, and BDNF were not significantly different in patients with JIA-associated uveitis in both those with inactive uveitis and those receiving systemic IMT, which indicates their possible dependency on the disease activity or the treatment (**Supplementary Figure 1**, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.39621/abstract).

Although only 1 patient with JIA-associated uveitis had active uveitis, the relative distribution of IL-29 (low in JIA-associated uveitis as compared to the other uveitis groups) was highly similar to that observed in patients with inactive uveitis (**Figure 2** and **Supplementary Figure 2**, online at http://onlinelibrary.wiley.com/doi/10.1002/art.39621/abstract). To further investigate this, multivariate logistic regression was performed for the JIA-associated uveitis group (n=21) versus the idiopathic uveitis group (n=29), taking into account uveitis activity, systemic IMT, and uveitis duration. The results revealed that the levels of IL-29/IFNλ1 were indeed significantly decreased in the JIA-associated uveitis group (odds ratio 0.61, [95% confidence interval (95% CI) 0.38-0.98]; $P=0.042$). The levels of IL-29 did not differ between patients with active and those with inactive arthritis (**Supplementary Figure 3**, online at http://onlinelibrary.wiley.com/doi/10.1002/art.39621/abstract).

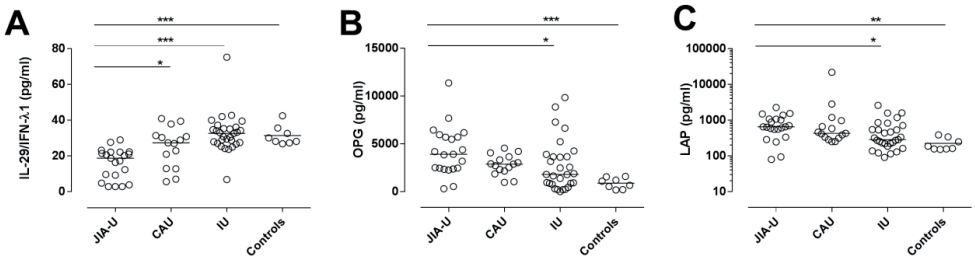

Figure 1. *Concentrations of the soluble mediators interleukin-29 (IL-29)/interferon-λ1 (IFNλ1) (**A**), osteoprotegerin (OPG) (**B**), and latency-associated peptide (LAP) (**C**) in the aqueous humor of patients with juvenile idiopathic arthritis (JIA)–associated uveitis (JIA-U), chronic anterior uveitis (CAU), idiopathic uveitis (IU), and noninflammatory conditions (controls). Each symbol represents an individual patient; horizontal lines show the median. *= P<0.05; **P<0.01; ***P<0.001 by Kruskal-Wallis test with Dunn's post hoc test.*

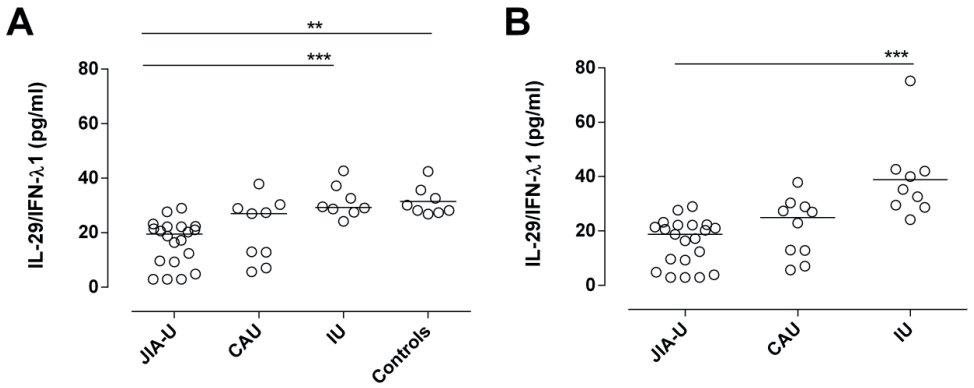

Figure 2. *Concentrations of IL-29/IFN λ1 in the aqueous humor of a subgroup of patients from the indicated study groups. **A**, Only patients with inactive uveitis. **B**, Only patients receiving systemic immunomodulatory therapy. Each symbol represents an individual patient; horizontal lines show the median. ** = P<0.01; *** = P<0.001 by Kruskal-Wallis test with Dunn's post hoc test. See **Figure 1** for definitions.*

Analysis of serum for soluble mediators

Thirty-seven of 51 soluble mediators were detected in the serum of the study patients (**Supplementary Table 2**). IL-29/IFN λ1 was detected in only 8 of 20 children with JIA-associated uveitis, 4 of 14 with chronic anterior uveitis, 6 of 25 with idiopathic uveitis, and 1 of 8 controls, and it was among the mediators that were excluded for further statistical comparison as described in Patients and Methods. In addition, the levels of LAP and OPG did not differ between the groups. Macrophage derived cytokine (MDC) levels were significantly decreased in the JIA-associated uveitis group as compared to the idiopathic uveitis and control groups, and IL-13 levels were increased in the JIA-associated uveitis group as compared to the idiopathic uveitis group (P=0.027 and P=0.042, respectively, for MDC; P=0.030 for IL-13). Also, MIP-3β levels were significantly decreased in JIA-associated uveitis patients as compared to the controls (P=0.003). No significant differences in serum levels between the JIA-associated uveitis and chronic anterior uveitis groups were found.

Analysis of childhood uveitis by visualization-based classification

We next used a projection-based visualization technique (Radviz) in which dimensions assigned to measured mediators in AqH were placed on the edge of a circle to examine the joint effect of multiple mediators. To explore meaningful patterns in Aqh, each AqH sample was assigned a unique location within the circle as a function of its relative attraction to each of these mediators. The results provide a visual representation of the joint effect of multiple biomarkers, with the aim of separating different forms of childhood uveitis. Although IL-29/IFNλ1 levels were lower in JIA-associated uveitis patients, no single analyte differentiated all 3 forms of childhood uveitis. However, analysis via VizRank sorted out meaningful insights into the underlying AqH profiles in the patients with childhood uveitis.

Figure 3 illustrates the best Radviz representation considering the simultaneous representation of 4 mediators. Top projections included the soluble mediators that were also found in univariate analyses, such as BDNF, IL-29/IFNλ1, OPG, and LAP (**Figure 3**). The overall projection accuracy for correctly classifying patients as having 1 of the 3 forms of childhood uveitis based on the of 4 soluble mediators in AqH was relatively low (~50%), with the best projection accuracy equal to 63.7% based on the levels of IL-15, IL-29/IFNλ1, IL-6, and soluble vascular cell adhesion molecule (**Figure 3**). Considering these mediators together revealed that JIA-associated uveitis and idiopathic uveitis are relatively well differentiated, with JIA-associated uveitis lying far from IL-29 (low levels of IL-29), in contrast to the positioning of idiopathic uveitis. As shown in **Figure 3**, chronic anterior uveitis mostly overlaps JIA-associated uveitis and thus contributes to the low projection accuracy for the 3 diseases. Indeed, head-to-head comparison of each group revealed that chronic anterior uveitis and JIA-associated uveitis are poorly differentiated (**Figure 3** and **Supplementary Figure 4**, online at http://onlinelibrary.wiley.com/doi/10.1002/art.39621/abstract), which suggests that there are related underlying protein patterns in AqH. In contrast, JIA-associated uveitis and idiopathic uveitis are accurately differentiated based on the 4 soluble mediators, with a best projection accuracy for correctly clasifying patients based on the levels of IL-29/IFNλ1, IL-22, angiotensin-converting enzyme, and IL-6 equal to 92.1%, indicating distinct profiles of soluble mediators in AqH.

Low levels of IL-29/IFNλ1 in JIA-associated uveitis

To explore the role of IL-29/IFNλ1 as a putative biomarker for JIA-associated uveitis, we performed ROC analyses, a useful tool for assessing biomarker accuracy.[21] ROC analysis

revealed that IL-29/IFNλ1 had an extremely high area under the curve (AUC) value (0.954 [95% CI 0.878-1.000]; specificity 97% and sensitivity 91%), with 23.5 pg/ml as the optimum cutoff value for correctly differentiating JIA-associated uveitis from idiopathic uveitis (**Figure 4**). Stratification according to systemic IMT (AUC = 0.989 [95% CI 0.947-1]) or inactive disease (AUC = 0.975 [95% CI 0.872-1]) improved the classification accuracy of IL-29/IFNλ1 (**Supplementary Figure 5**, online at http://onlinelibrary.wiley.com/doi/10.1002/art.39621/abstract). The ROC analysis for IL-29/ IFNλ1 showed a moderate prediction of JIA-associated uveitis versus chronic anterior uveitis (AUC 0.771 [95% CI 0.594-0.916]; specificity 67% and sensitivity 86%), with a comparable optimum cutoff value of 22.7 pg/ml. Stratification for systemic IMT and inactive disease slightly decreased the AUC values (0.705 [95% CI 0.447-0.876] and 0.672 [95% CI 0.437-0.878], respectively) (**Supplementary Figure 5**). Tenfold cross-validation and bootstrap resampling (n=2,000) analyses generated similar results.

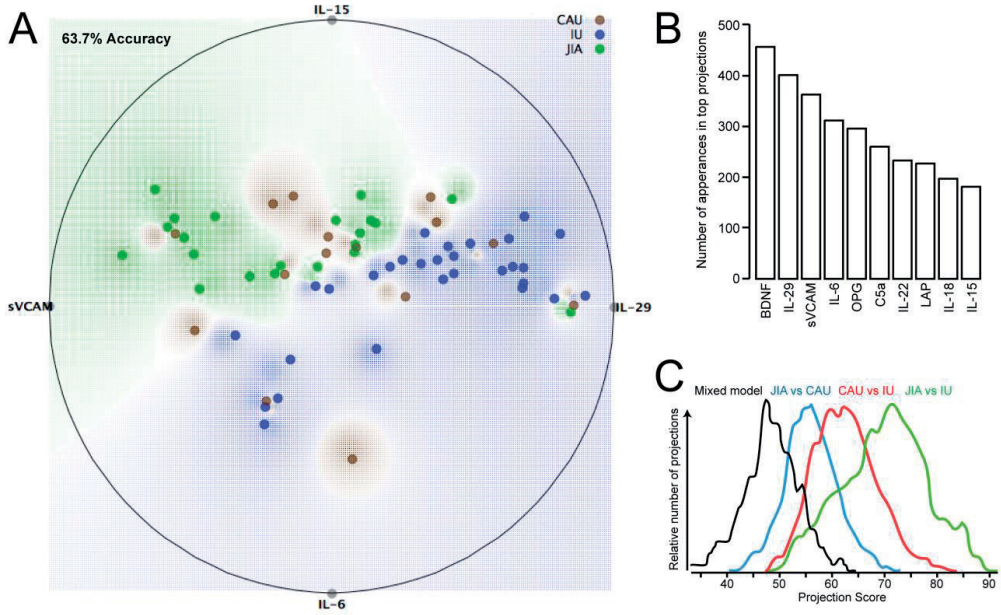

Figure 3. *Visualization of the joint effect of 4 soluble mediators present in ocular fluid that have the best capacity for distinguishing 3 forms of childhood uveitis. The radial coordinate visualization method (VizRank method; Radviz) was used to project high-dimensional data into the orthogonal space.* ***A****, Of 5,000 projections, the best projection accuracy for distinguishing the 3 forms of uveitis had a value of 63.7%, based on the levels of IL-15, IL-29/IFN-λ1, IL-6, and soluble vascular cell adhesion molecule (sVCAM).* ***B****, The top 10 most abundant soluble mediators in top projections were determined by the Radviz method.* ***C****, The overall projection scores for the mixed model and direct comparisons of the overall projection scores for each of the uveitis groups are shown. BDNF = brain-derived neurotrophic factor (see* ***Figure 1*** *for other definitions).*

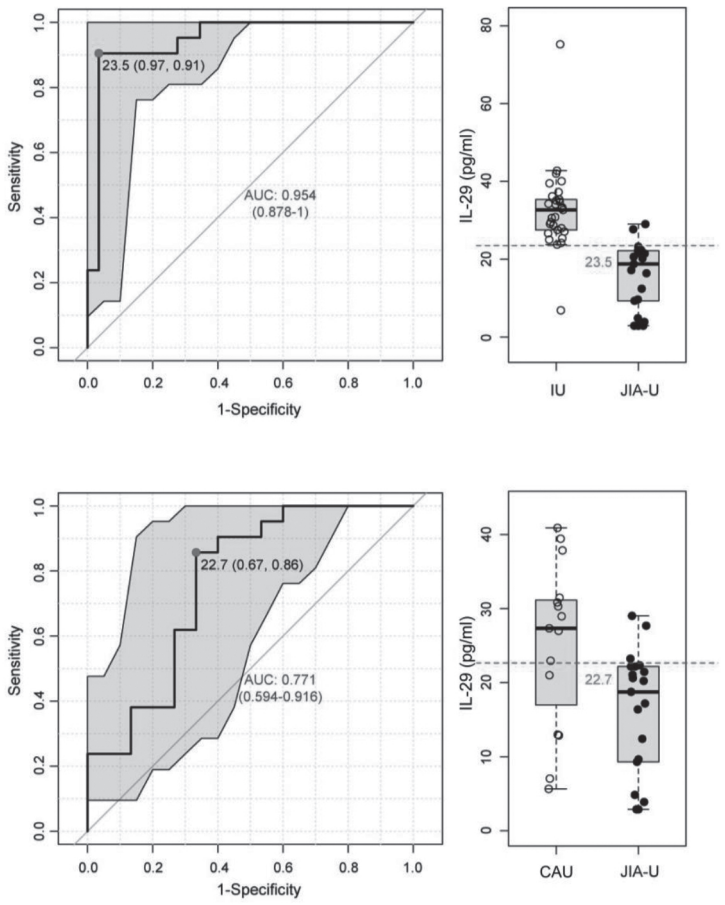

Figure 4. *Receiver operating characteristic (ROC) curve analysis of IL-29/IFN-λ1 levels. ROC analyses of the IL-29/IFN-λ1 levels were performed in all patients with JIA–associated uveitis versus idiopathic uveitis (top) and versus chronic anterior uveitis (bottom). Each ROC curve includes a 95% confidence interval (shaded area). The numbers at the left upper corner represent the optimum cutoff value for correctly differentiating JIA-associated uveitis from idiopathic uveitis, which is represented by a horizontal dotted line in the separate scatterplot of the IL-29 levels in each patient in the 2 groups (right panels). Numbers in parentheses at the curve represent the specificity and sensitivity, respectively, of the model. The area under the curve (AUC) values are shown with 95% confidence intervals. The scatterplot data are also shown as box plots. Each box represents the 25th to 75th percentiles. Lines inside the boxes represent the median. Lines outside the boxes represent the 10th and 90th percentiles. See* ***Figure 1*** *for other definitions. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/journal/doi/10.1002/art.39621/abstract.*

DISCUSSION

In this study, we identified IL-29/IFNλ1 in AqH as a potential intraocular biomarker for JIA-associated uveitis as compared to other forms of non-infectious childhood uveitis. The overall ocular mediator profiles of JIA-associated uveitis and idiopathic childhood uveitis (idiopathic uveitis) differed. In contrast, chronic anterior uveitis and JIA-associated uveitis, in addition to their clinical resemblance, showed much more similar soluble mediator profiles.

IL-29 (more commonly known as IFNλ1) is the main cytokine of the family of type III interferons that includes 3 λ-family members in humans (IFNλ1-3). The IFNλ family members signal through their receptor IFNLR1/IL-28R expressed by epithelial and immune cells.[22,23] Although plasmacytoid dendritic cells are potent producers of IL-29/IFNλ1 in response to a viral infection, other cell types, including epithelial cells, mast cells, and Th17 cells have been shown to secrete IL-29.[24-28] IFNλ is also very effective at viral clearance independently of adaptive immunity.[29] While essential to viral control, bacterial infection can also induce potent expression of IFNλ.[30]

Currently, the mode of action of the relatively unexplored IFNλ family is ambiguous and probably pleotropic, but it is thought to be mainly related to the regulation of the immune cell-epithelial cell interface. The IFNλ family can function in a synergistic role or an immunomodulatory role, distinct from IFNα/β signaling.[31-34] Although the exact causal mechanisms most likely include a myriad of factors, we provide herein evidence of a role of decreased intraocular levels of IFNλ1 in patients with JIA-associated uveitis.

Recent insights have shown that IFNλ1 is crucial for tightening the region-specific blood-brain barrier permeability by regulating microvascular endothelial cells to inhibit viral infection[24] The IFN-λ are expressed in human eye, and it is tempting to speculate that they also regulate the permeability of the retina-ocular barrier.[35] The decreased levels of IL-29/IFNλ1 levels may be related to insufficient barrier function in JIA-associated uveitis and may facilitate opportunistic or concealed viral infection. Curiously, recent studies showed that >50% of patients with JIA-associated uveitis had high titers of anti-parvovirus B19 antibody in AqH, as compared to <10% of patients with other forms of uveitis.[8,36-38] Impaired production of antiviral IL-29/IFNλ1 and increased parvovirus B19 in ocular epithelial cells may be one of the underlying mechanisms that lead to JIA-associated uveitis.

Interestingly, IL-29/IFNλ1 was previously shown to be elevated in the serum of patients with rheumatoid arthritis (RA) (mean ± SD 24.56 ± 15.85 pg/ml, in the same range as we found in patients with chronic anterior uveitis and idiopathic uveitis) and to enhance the cytokine responses of synovial fibroblasts.[39] RA, however, is a different entity, since our study patients had rheumatoid factor-negative oligo- and polyarthritis. We did not detect IL-29/IFNλ1 in the serum of patients with JIA-associated uveitis, and we observed decreased levels in their AqH, indicating distinct and probably site-specific effects of IFNλ in JIA-associated uveitis as compared to RA. Also in contrast to RA, which displayed an association of serum IFNλ1 with arthritis[40], the intraocular IL-29/IFNλ1 levels in JIA-associated uveitis did not correlate with arthritis activity (**Supplementary Figure 3**). Paradoxically, the functionally related IFNλ family member IL-28A resolves collagen-induced arthritis in mice.[23]

Thus, the exact functional implication of the recently emerging IFNλ family in various rheumatic diseases is not yet conclusive, is probably pleotropic, and is different between the targeted issue(s). Although we did not measure any other family member of the type III interferons, their close relationship to barrier function during viral infection and the previous reports on parvovirus B19 in JIA-associated uveitis warrant further detailed research to

elucidate the role of this intriguing interferon family in the pathogenesis of JIA-associated uveitis.[41]

In addition to IL-29, we found LAP and OPG to be specifically increased for JIA-associated uveitis as compared to those with idiopathic uveitis and as compared to the controls, but after correction for disease activity and systemic treatment, the difference was lost. LAP is a latent form of transforming growth factor β (TGFβ) and inhibits the biological activity of TGFβ, which itself has various immune regulatory effects. Increased LAP levels could influence the balance of T helper and T regulatory cells in JIA.[8] OPG functions in bone tissue by inhibiting the maturation of osteoclasts. OPG appeared to be slightly increased in the serum of patients with JIA-associated uveitis.[42] The role of LAP and OPG in JIA-associated uveitis is uncertain and may reflect processes related to the arthritis (JIA) in these patients.

The levels of most other soluble mediators were similar between JIA-associated uveitis, chronic anterior uveitis, and idiopathic uveitis groups and were consistent with previous observations. AqH analysis in 11 JIA-associated uveitis patients conducted by Sijssens and colleagues[43] did not indicate any differences from other forms of childhood uveitis. Thus, a classification model that takes into account multiple soluble mediators is needed in order to reveal in greater detail the delicate differences between these uveitis entities.

In this study, idiopathic uveitis and JIA-associated uveitis could be distinguished very well by cytokine profiles, but we found that this was predominantly dependent on the IL-29 levels. JIA-associated uveitis and chronic anterior uveitis (without arthritis) have similar clinical manifestations and, thus, chronic anterior uveitis has been suggested to be the first extraarticular manifestation of JIA.[44] Indeed, among patients in the heterogeneous group of chronic anterior uveitis (based upon exclusion of systemic disease and the absence of involvement of other parts of the eye) few may develop JIA. In fact, 3 patients in the JIA-associated uveitis group were initially diagnosed as having chronic anterior uveitis, having presented with uveitis before the onset of arthritis. Strikingly, these patients also had higher levels of IL-29 in AqH at the time of study sampling (median 31.5 pg/ml [range 30.8-39.4]), which indicates that the decrease in IL-29 levels may somehow be related to the presence of arthritis. The difference in the levels of IL-29/IFNλ1 between the JIA-associated uveitis and chronic anterior uveitis groups was lost after correcting for disease activity and treatment, perhaps due to loss of power, since the median levels of IL-29/IFNλ1 did not change significantly, or perhaps because the levels of IL-29/IFNλ1 in chronic anterior uveitis are in fact very similar to those in JIA-associated uveitis, which may parallel their clinical similarity.

Given the clinical resemblance and the possible relationship between chronic anterior uveitis and JIA-associated uveitis, we tried to investigate this in more detail by modelling combinations of multiple mediators in AqH to reveal additional subtle changes in cytokine profiles. However, the models of the soluble mediator in AqH revealed that chronic anterior uveitis was a heterogeneous group, with characteristics of both idiopathic uveitis and JIA-associated uveitis attributing to the difficulty of distinguishing the 3 diseases. Head-to-head comparison of multicytokine profiles revealed that chronic anterior uveitis could not be distinguished from JIA-associated uveitis (**Supplementary Figure 4A**). This is consistent with the observation by Kalinina Ayuso et al[45] that that the proteomic fingerprints of chronic anterior uveitis and JIA-associated uveitis are very similar.

There are several important points to be made with regard to the results of this study. For example, although the levels IL-29/IFNλ1 expression expression can distinguish major forms of childhood uveitis, they do not predict whether a child with JIA carries a higher risk of developing uveitis. Ideally, an exploratory analysis similar to the present study should be

performed in AqH samples from JIA patients with and without uveitis. AqH taps, however, are generally not done in children who do not have clinical signs of uveitis, which makes such a research proposal unethical. To answer such a question, other stratified population studies with JIA patients are needed. In a recent study[46], we identified an elevated erythrocyte sedimentation rate at JIA onset to be a valuable and accessible indicator of the development of uveitis in JIA patients. Thorough whole-genome analysis of JIA-associated uveitis versus JIA without uveitis may also provide high-risk alleles, perhaps underlying the IL-29 pathway, that may be useful in future genetic screening tools for JIA patients. Although serum analysis may be more accessible compared to ocular fluid analysis, the protein composition of serum did not reflect the local ocular characteristics of each mediator and was less informative than the AqH analysis in this study, indicating that local pathologic mechanisms involving IL-29/IFNλ1 contribute significantly to the development of JIA-associated uveitis.

The additional inclusion of patients with JIA who were experiencing their first episode of uveitis activity and were not receiving systemic treatment at that time would have bene ideal. However, AqH samples are rarely obtained from children, and establishment of the diagnosis of JIA-associated uveitis commonly does not require AqH sampling. More importantly, uveitis typically develops after arthritis and the accompanying use of systemic IMT. Since JIA-associated uveitis often leads to complications that warrant surgery (e.g., cataracts or glaucoma), systemic IMT is also needed to suppress disease activity in order to adequately perform the surgery. This results in an experimental setup where the majority of JIA-associated uveitis samples are collected during the chronic, inactive stage of disease, whereas many of the samples from the comparator diseases were collected from more actively inflamed eyes, which could potentially affect the outcome of the study.

To thoroughly address this issue, we normalized the AqH data by specifically comparing only patients with inactive uveitis or only patients receiving systemic IMT. Both univariate and multivariate analyses correcting for disease activity, systemic treatment, and uveitis duration revealed that IL-29/IFNλ1 were much lower in JIA-associated uveitis group as compared to the idiopathic uveitis group, with hardly any overlap. Although this would suggest that IL-29/IFNλ1 levels are independent of disease activity or systemic IMT, it is possible that the sample size and power were simply too low to detect subtle differences. In addition, given the observational nature of this study, it is not possible to assess whether lower intraocular levels of IL-29 are a cause or result of JIA-associated uveitis. It would be interesting to include samples obtained at disease onset in upcoming validation studies, for which the findings of this unprecedented cohort study have paved the way for understanding the type III interferon family in JIA-associated uveitis.

We would emphasize that the lack of disease specificity of other soluble mediators in this study does not mean that they do not play a significant role in its pathophysiology. Therapy with anti-TNF (adalimumab) and anti-IL-6 (tocilizumab), for example, has been shown to effectively control uveitis in some JIA patients.[47,48] Nevertheless, in a significant proportion of children with JIA-associated uveitis, most conventional treatments are either inadequate or frequently accompanied by severe adverse effects. The findings of the current study therefore add to the understanding of JIA-associated uveitis and may contribute to the development of novel targeted therapies for this group of children.

In summary, we identified IL-29/IFNλ1 as an intraocular biomarker for JIA-associated uveitis. This finding suggests that aberrant IFNλ signaling might be important in the uveitis associated with JIA.

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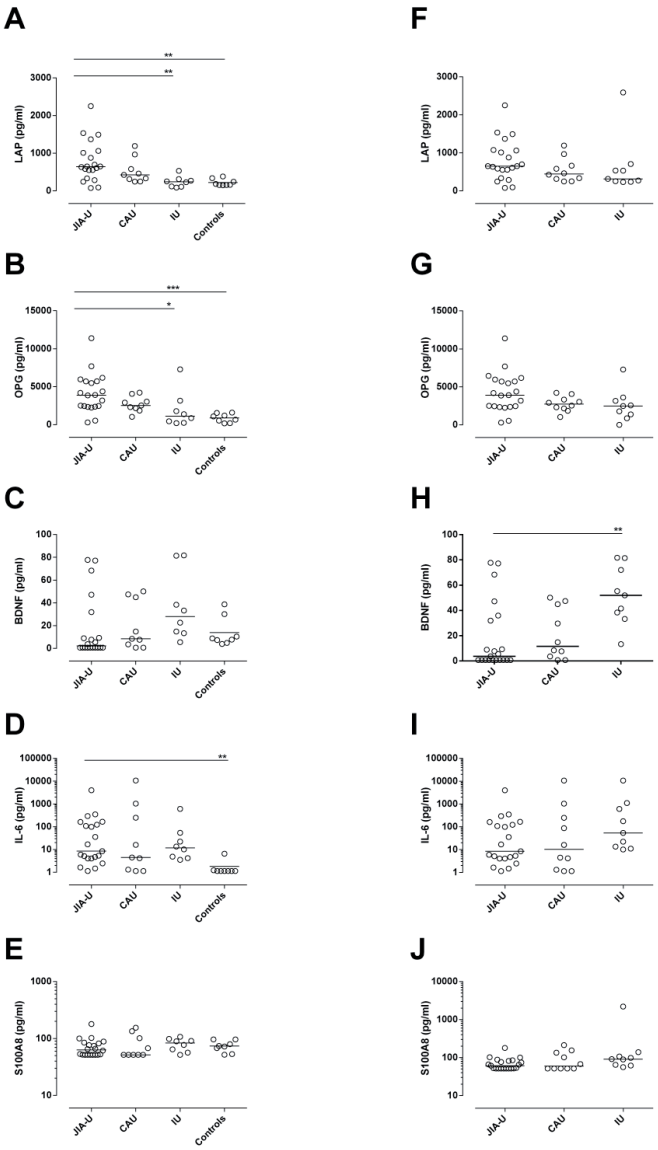
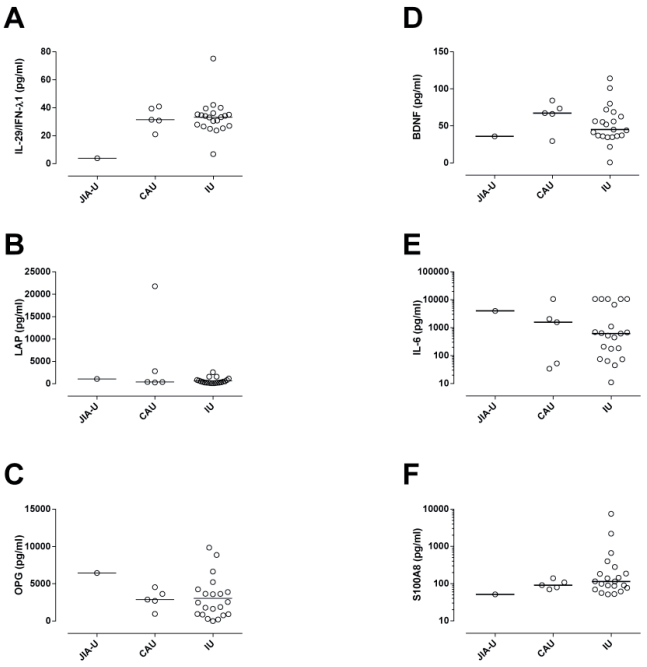
SUPPLEMENTARY FIGURES AND TABLES



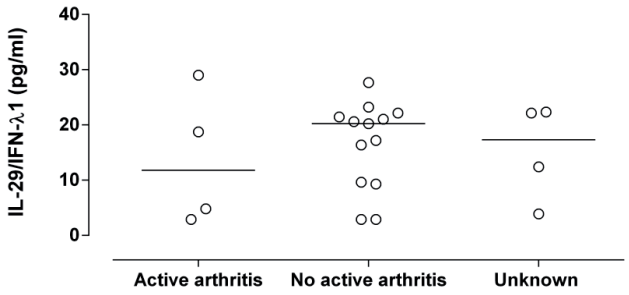
Supplementary Figure 1.*Concentrations of several mediators in the aqueous humor of patients with (A-E) inactive uveitis and (F-J) on systemic immunomodulatory treatment at the moment of sampling. The horizontal line per patient group represents the median concentration of the mediator. Data were analyzed by the Kruskal-Wallis test with post hoc Dunn's test. *= significant outcome with *P<0.05, **P<0.01 and ***P<0.001.*

JIA-U = uveitis associated with juvenile idiopathic arthritis; CAU = chronic anterior uveitis; IU = idiopathic uveitis; Controls = non-inflammatory controls; LAP = latency associated peptide; OPG = osteoprotegerin; BDNF = brain-derived neurotrophic factor; IL = Interleukin; S100A8 = S100 calcium binding protein A8

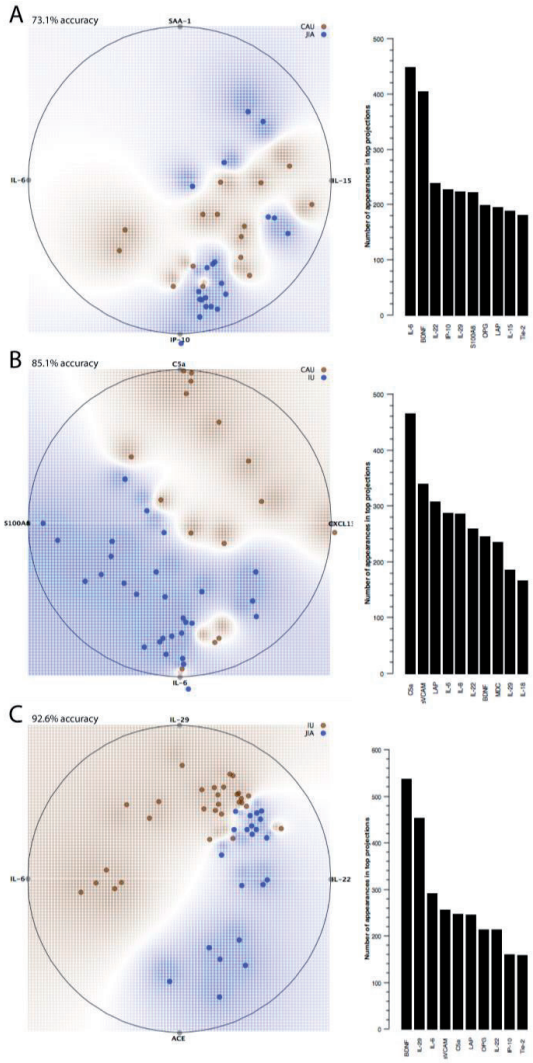


Supplementary Figure 2. *Concentrations of several mediators in the aqueous humor of patients with active uveitis. The horizontal line per patient group represents the median concentration of the mediator. Data were analyzed by the Kruskal-Wallis test with post hoc Dunn's test. There were no significant outcomes.*

JIA-U = uveitis associated with juvenile idiopathic arthritis; CAU = chronic anterior uveitis; IU = idiopathic uveitis; Controls = non-inflammatory controls; IL-29 = interleukin 29; IFN-λ1 = interferon-λ1; LAP = latency associated peptide; OPG = osteoprotegerin; BDNF = brain-derived neurotrophic factor; IL = Interleukin; S100A8 = S100 calcium binding protein A8

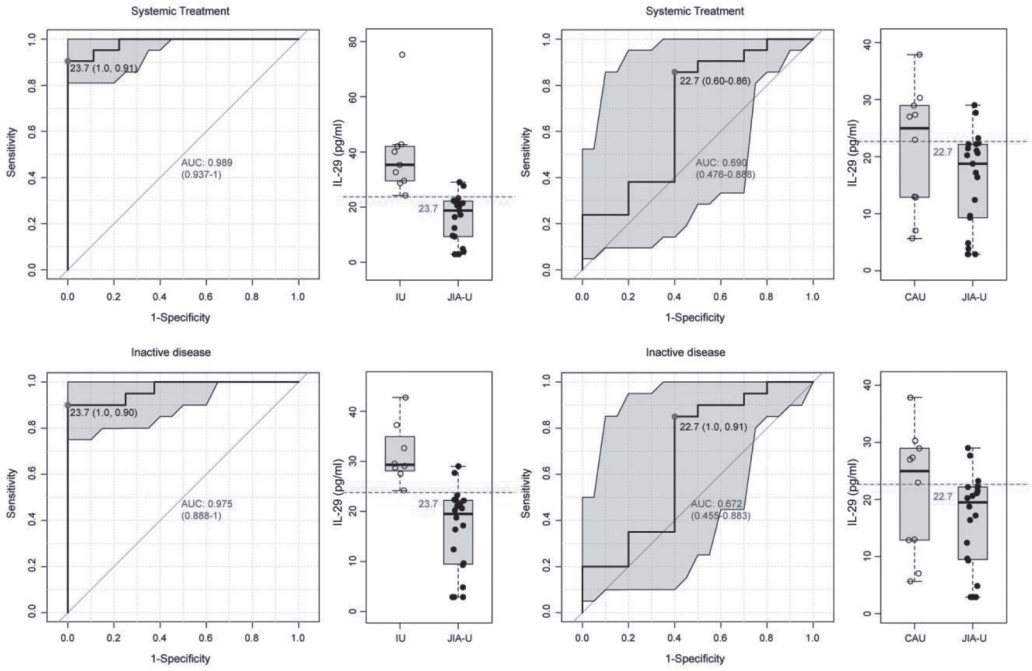


Supplementary Figure 3. *Concentrations of the mediator interleukin 29 (IL-29)/interferon-λ1 (IFN- λ1) in the aqueous humor of patients with uveitis associated with juvenile idiopathic arthritis (JIA-U) according to arthritis activity. The horizontal line per patient group represents the median concentration of the mediator. Data were analyzed by the Kruskal-Wallis test. There were no significant differences. IL= interleukin; IFN = interferon*



Supplementary Figure 4. *Visualization of the joint effect of four soluble mediators in aqueous humor with the best capacity to distinguish between childhood uveitis. The radial coordinate visualization method (VizRank method, Radviz™) was exploited to represent high dimensional data into the orthogonal space. (a) Best projection of head-to-head comparison of JIA-U versus CAU (left panel) and top 10 most abundant soluble mediators in top projections. (b) Best projection of head-to-head comparison of CAU versus IU (left panel) and top 10 most abundant soluble mediators in top projections. (c) Best projection of head-to-head comparison of JIA-U versus IU (left panel) and top 10 most abundant soluble mediators in top projections.*

JIA-U = uveitis associated with juvenile idiopathic arthritis; CAU = chronic anterior uveitis; IU = idiopathic non-infectious childhood uveitis; IL= interleukin; sVCAM = soluble vascular cell adhesion protein, ACE = angiotensin converting enzyme, IP = interferon gamma-induced protein, SAA = serum amyloid A, S100A8 = S100 calcium binding protein A8, CXCL = chemokine (C-X-C motif) ligand, C5a = complement component 5a



Supplementary Figure 5. *Receiver operating characteristic (ROC) curve of IL-29/IFN-λ1. ROC analysis of IL-29/IFN-λ1 in Uveitis associated with juvenile idiopathic arthritis (JIA-U) versus idiopathic uveitis (IU) versus and JIA-U versus chronic anterior uveitis (CAU). Separate analysis was done including patients using systemic treatment (Systemic treatment, top), and patient with inactive disease (Inactive disease, bottom). Each ROC curve is accompanied by a 95% confidence interval (blue) and a scatterplot of the analysed cases including a redline indicating the optimal cut-off value in pg/ml.*

Supplementary Table 1. Mediators in *aqueous humor* of patients with juvenile idiopathic arthritis associated uveitis, chronic anterior uveitis without arthritis, idiopathic uveitis and non-inflammatory controls

Mediator	JIA-U (n=21)	CAU (n=15)	IU (n=29)	Controls (n=8)		JIA-U vs CAU	JIA vs IU	JIA-U vs controls
	Median Range				*p-value*[c]	*p-value's*[d]		
IL-29[a]	18.8	27.3	32.7	29.1	**<0.001**	**0.015**	**<0.001**	**<0.001**
	(2.9-29.0)	(5.7-40.9)	(6.9-75.2)	(26.8-42.5)				
LAP[a]	651.1	427.1	283.8	174.1	**0.002**	1.000	**0.036**	**0.003**
	(79.7-2250.2)	(249.2-21796.0)	(90.9-5-2589.0)	(152.3-385.4)				
OPG[a]	3907.1	2876.6	1794.7	874.9	**0.001**	0.744	**0.030**	**<0.001**
	(298.6-11377.6)	(971.7-4550.7)	(5.5-9856.0)	(180.4-1584.5)				
IL-6[a]	8.5	52.3	209.2	1.1	**<0.001**	0.900	**0.018**	**0.027**
	(1.1-3998.5)	(1.1-10684.6)	(3.5-10684.6)	(1.1-6.6)				
BDNF[a]	3.7	29.7	41.5	8.3	**<0.001**	0.105	**<0.001**	1.000
	(0.7-77.7)	(0.7-84.4)	(0.7-114.3)	(3.9-38.7)				
S100A8[a]	61.4	79.5	90.9	73.2	**0.010**	0.432	**0.003**	1.000
	(51.7-179.9)	(51.7-213.0)	(51.7-7540.8)	(52.1-96.5)				
IL-18[a]	6.7	5.3	3.4	1.8	**0.020**	0.927	0.222	**0.006**
	(0.7-750.2)	(5.3-39.1)	(0.7-37.9)	(0.7-5.7)				
MIP-3β/CCL19[a]	9.0	14.3	13.2	0.7	**0.003**	1.000	1.000	**0.003**
	(0.7-3547.7)	(0.7-1420.2)	(0.7-1500.6)	(0.7-2.5)				
MDC/CCL22[a]	41.9	24.8	25	4.9	**0.017**	1.000	1.000	**0.009**
	(0.9-1049.8)	(11.1-478.5)	(0.9-370.0)	(0.9-20.4)				
sICAM[a]	11447.6	11602.6	5881.0	928.8	**0.001**	1.000	1.000	**<0.001**
	(1390.8-65213.9)	(1634.6-61227.4)	(7.3-55410.3)	(292.9-3554.9)				
MIG/CXCL9[a]	28.4	29.13	30.5	13.5	**0.014**	1.000	1.000	**0.009**
	(3.6-4852.8)	(10.7-718.4)	(6.4-2214.6)	(7.3-18.7)				
BLC[a]	40.6	45.71	12.9	0.7	**0.001**	1.000	0.720	**<0.001**
	(0.7-9803.4)	(0.7-8537.4)	(0.7-8921.4)	(0.7-1.0)				
sVCAM[b]	28.5	28.9	16.9	2.5	**0.001**	1.000	0.474	**<0.001**
	(4.6-63.3)	(4.3-82.3)	(2.1-97.9)	(0.9-18.1)				
sCD14[b]	282.2	122.4	211.7	10.9	**<0.001**	1.000	1.000	**<0.001**
	(12.7-282.2)	(42.0-282.2)	(9.2-282.2)	(4.1-48.5)				
TIE-2/TEK[a]	1640.8	1565.7	1187.99	174.1	**0.002**	1.000	1.000	**<0.001**
	(172.7-6419.2)	(122.2-2784.2)	(27.2-4557.0)	(31.8-970.5)				

C5a[a]	191.0	170.8	44.8	3.9	**<0.001**	1.000	0.054	**<0.001**
	(2.4-3812.3)	(2.42-13419.5)	(2.4-1285.5)	(2.4-37.3)				
ACE[a]	5166.3	3310.7	2694.21	625.2	**0.001**	1.000	0.390	**<0.001**
	(531.1-50001.8)	(937.4-15495.5)	(578.7-28787.2)	(265.4-2636.8)				
IL-22[a]	48.6	52.22	38.0	68.9	**0.001**	0.558	0.282	0.063
	(18.3-68.8)	(52.2-4358.9)	(1.7-178.0)	(42.4-112.2)				
IL-8/CXCL8[a]	13.7	18.22	93.4	1.3	**<0.001**	1.000	0.054	**0.012**
	(1.3-358.6)	(1.7-2201.3)	(1.3-3211.7)	(1.3-7.4)				
SAA-1[a]	897.1	1361.8	1917.5	497.5	**0.026**	0.366	0.150	0.780
	(370.3-16115.5)	(370.3-8875.3)	(370.3-54815.9)	(370.3-1166.1)				
IP-10/CXCL10[a]	2611.0	1678.0	2715.2	97.5	0.062			
	(1.6-5455.4)	(88.5-5455.4)	(15.9-5455.4)	(6.2-5455.4)				
IL-15[a]	42.3	42.31	44.2	31.5	0.061			
	(18.1-103.3)	(42.3-184.0)	(20.2-100.9)	(29.6-39.1)				
IL-21[a]	1007.9	1064.8	1119.1	870.3	0.071			
	(301.9-1927.6)	(1064.8-14509.4)	(545.9-2283.8)	(543.2-1154.5)				
IL-5[a]	6.9	7.2	7.7	8.0	0.507			
	(0.7-11.7)	(3.8-17.2)	(2.9-40.0)	(4.2-9.5)				
IL-27[a]	1564.9	42.3	1707.7	1532.3	0.678			
	(881.9-8859.1)	(1152.6-14211.0)	(1090.1-4264.1)	(1144.2-1924.9)				
TPO[a]	6494.2	5535.1	6936.4	5732.0	0.174			
	(574.8-18071.0)	(574.8-17195.1)	(2297.3-27878.4)	(1099.5-7059.1)				
Amphiregulin[a]	4.2	4.3	4.4	4.3	0.685			
	(1.7-6.1)	(3.3-7.0)	(3.5-7.2)	(4.1-5.7)				
NGF[a]	6.0	6.6	6.4	7.1	0.195			
	(1.2-8.9)	(4.6-8.8)	(3.7-11.7)	(4.3-8.3)				
VEGF[a]	106.1	120.8	128.5	49.4	0.151			
	(9.4-250.7)	(32.2-384.6)	(5.4-2796.7)	(10.7-124.2)				
sIL-2R[a]	318.5	463.3	257.5	154.1	0.243			
	(9.3-3098.5)	(139.5-6133.1)	(54.4-8930.9)	(109.4-5565.1)				
Ang-2[a]	191.5	281.7	239.7	68.9	0.392			
	(5.5-1420.3)	(13.9-831.4)	(4.9-1619.9)	(8.6-1132.3)				
Eotaxin/CCL11[a]	8.1	7.9	7.5	7.6	0.302			
	(6.1-13.6)	(7.1-24.8)	(6.0-30.4)	(7.1-8.7)				
MCP-1/CCL2[a]	187.6	211.6	382.9	159.7	0.085			
	(51.4-651.5)	(96.9-1582.9)	(38.4-1161.8)	(66.0-250.5)				
MIF[a]	3669.5	3881.3	4380.7	6996.4	0.784			

	(761.4-61109.5)	(586.0-57560.6)	(925.7-79936.9)	(1294.3-10930.5)		
IL-26[a]	134.1	110.8	126.2	97.7	0.629	
	(25.6-510.7)	(22.7-610.7)	(22.7-610.7)	(30.4-148.0)		

[a]concentration of the mediator in pg/ml
[b]concentration of the mediator in ng/ml
[c]p-values derived from Kruskal-Wallis analysis
[d]p-values derived from post-Kruskal-Wallis Dunn's test
AqH of patients with different non-infectious uveitis entities as well as non-inflammatory controls was measured for different mediators. Data of 35 mediators are presented as median with their corresponding range. Significant p-values are highlighted in bold.
AqH = aqueous humor, JIA-U = uveitis associated with juvenile idiopathic arthritis, vs = versus, CAU = chronic anterior uveitis without arthritis, IU = idiopathic uveitis, Controls = non-inflammatory controls, IL = interleukin, IFN = interferon LAP = latency associated peptide, TGF = transforming growth factor, MIP = macrophage inflammatory protein, CCL = chemokine (C-C motif) ligand, MDC = macrophage derived cytokine, CXCL = chemokine (C-X-C motif) ligand, MIG = monokine induced by gamma interferon, BLC = B lymphocyte chemoattractant, OPG = osteoprotegerin, TPO = thrombopoietin, SAA = serum amyloid A, NGF = nerve growth factor, BDNF = brain-derived neurotrophic factor, VEGF = vascular endothelial growth factor, sICAM = soluble intracellular adhesion molecule, sVCAM = soluble vascular cell adhesion protein, CD = cluster of differentiation, sCD14 = soluble CD14, S100A8 = S100 calcium binding protein A8, MRP = myeoloid-related protein, sIL-2R = soluble IL-2 receptor, Ang = angiopoietin, TIE = tyrosine kinase with immunoglobulin-like and EGF-like domains, TEK = tunica endothelial kinase, C5a = complement component 5a, MCP = monocyte chemoattractant protein, IP = interferon gamma-induced protein, MIF = macrophage migration inhibitory factor, ACE = angiotensin converting enzyme.

Supplementary Table 2. Mediators in *serum* of patients with juvenile idiopathic arthritis associated uveitis, chronic anterior uveitis without arthritis, idiopathic uveitis and non-inflammatory controls

Mediator	**JIA-U (n=20)**	**CAU (n=14)**	**IU (n=25)**	**Controls (N=7)**		**JIA-U vs CAU**	**JIA-U vs IU**	**JIA-U vs controls**
	Median Range				*p-value*[c]	*p-value's*[d]		
LAP[a]	32846.2	29600.3	30107.4	26994.2	0.557			
	(13581.8-45641.8)	(22096.4-42600.0)	(17175.2-57132.6)	(19378.8-43001.9)				
OPG[a]	386.7	353.3	455.1	653.4	0.492			
	(90.4-1074.4)	(4.8-796.3)	(4.8-885.7)	(178.7-740.5)				
IL-6[a]	5.8	5.6	3.0	38.8	0.357			
	(1.2-259.0)	(1.2-187.0)	(1.2-211.0)	(1.2-61.0)				
BDNF[a]	151700.7	140959.1	134335.0	119178.8	0.359			
	(59225.0-210528.2)	(109871.9-188716.3)	(74544.5-206797.8)	(95431.8-223808.9)				
IL-18[a]	147.4	180.9	108.0	197.5	**0.033**	0.738	0.264	1.000
	(42.1-332.8)	(70.1-605.8)	(35.0-551.9)	(89.4-386.1)				
MIP-3β/CCL19[a]	30.1	33.1	41.6	117.4	**0.012**	1.000	1.000	**0.003**
	(0.6-134.6)	(5.7-111.4)	(2.5-165.8)	(35.6-351.9)				
MDC/CCL22[a]	421.6	760.7	736.9	769.8	**0.017**	0.066	**0.027**	**0.042**
	(82.3-1271.0)	(188.3-2464.7)	(150.6-1938.4)	(339.8-1929.0)				
sICAM[a]	555787.9	622992.8	592743.8	849404.1	0.067			
	(389656.4-842960.5)	(305277.2-1060000.0)	(350740.0-1320000.0)	(498522.7-1020000.0)				
MIG/CXCL9[a]	51.7	73.7	47.3	49.8	0.675			
	(25.2-391.1)	(25.7-132.4)	(5.3-580.4)	(10.9-333.4)				
BLC[a]	36.6	51.8	16.7	66.0	0.085			
	(2.7-88.1)	(8.6-216.9)	(4.0-129.6)	(7.0-169.0)				
sVCAM[b]	2125.0	2330.0	2240	3230.0	**0.034**	1.000	1.000	0.093
	(1150.0-4290.0)	(1280.0-3520.0)	(995.9-3940.0)	(1990.0-5020.0)				
sCD14[b]	1425.0	1300.0	1400.0	1640.0	0.657			
	(434.0-2420.0)	(880.5-2330.0)	(599.2-4710.0)	(686.0-2820.0)				
TIE-2/TEK[a]	7529.7	7404.5	8580.0	8842.1	0.778			
	(3741.3-12322.6)	(5341.5-13820.8)	(4961.7-14307.4)	(5234.0-12394.4)				
C5a[a]	21389.9	19846.2	22375.4	24907.9	0.765			
	(4941.8-2100000.0)	(10879.2-30121.1)	(13589.0-237695.8)	(9999.7-78375.1)				
ACE[a]	191348.0	218660.4	179551.8	168467.8	0.826			
	(95518.3-375417.5)	(111381.6-356722.4)	(114421.8-376676.7)	(100358.3-350493.9)				
IL-22[a]	6.6	6.8	12.2	30.1	0.187			

	(1.1-214.0)	(1.1-57.8)	(1.1-178.0)	(1.1-85.4)				
IL-8/CXCL8[a]	70.2	53.1	19.7	72.3	0.099			
	(6-442.8)	(8.4-275.3)	(6.2-519.0)	(10.0-849.1)				
SAA-1[a]	545991.2	593563.4	525156.2	661479.2	0.762			
	(273692.2-1540000.0)	(422724.1-1230000.0)	(228626.8-1600000.0)	(260914.3-813901.8)				
IP-10/CXCL10[a]	109.6	177.7	200.9	413.3	0.119			
	(11.8-1383.9)	(43.8-2186.3)	(22.3-1461.7)	(50.6-1855.5)				
IL-15[a]	151.5	155.5	112.8	103.7	0.161			
	(90.3-288.6)	(68.9-327.3)	(48.0-419.8)	(48.0-412.4)				
IL-21[a]	5140.2	9662.9	1472.9	8558.8	**0.019**	0.678	0.147	1.000
	(349.4-43108.2)	(846.2-9330135)	(268.7-16452112.0)	(161.4-15895302.0)				
IL-23 p19[a]	88.5	412.4	8.3	54.9	**0.011**	0.567	0.126	1.000
	(8.3-3026.9)	(8.3-5823.3)	(8.3-8067.9)	(8.3-2675.6)				
IL-13[a]	101.6	130.6	26.4	81.7	**0.006**	1.000	**0.030**	1.000
	(17.7-619.1)	(32.0-974.8)	(5.3-1311.8)	(1.4-1073.3)				
IL-5[a]	12.4	16.4	9.4	15.7	0.934			
	(2.7-223.0)	(3.5-112.6)	(1.1-198.0)	(3.1-127.4)				
IL-27[a]	2002.4	2098.5	1602.2	1470.0	0.936			
	(167-10415.5)	(167-9852.8)	(167.0-10653.0)	(167.0-2822128.0)				
TSLP[a]	2.9	5.1	1.5	9.8	0.285			
	(0.4-38.4)	(0.5-50.5)	(0.1-76.3)	(0.1-98.9)				
Amphiregulin[a]	15.1	19.9	5.8	13.2	0.206			
	(3.1-75.0)	(3.1-91.0)	(3.1-126.4)	(3.1-170.0)				
VEGF[a]	106.0	117.2	91.3	184.4	0.552			
	(19.2-1135.2)	(11.9-269.5)	(12.8-373.1)	(18.9-440.6)				
sIL-2R[a]	540.4	678.6	487.7	1186.8	0.140			
	(15.0-978.0)	(272.0-1440.8)	(214.5-1519.9)	(80.1-2311.4)				
Ang-1[b]	69.2	69.0	66.1	74.0	0.973			
	(6.1-134.2)	(27.7-10.4)	(8.3-119.0)	(35.4-103.2)				
Ang-2[a]	964.6	933.8	922.8	1531.4	0.199			
	(135.9-2771.1)	(314.5-3267.8)	(251.7-4368.2)	(584.7-2952.0)				
Eotaxin/CCL11[a]	41.8	42.1	31.5	52.3	0.184			
	(9.9-102.1)	(11.0-75.9)	(4.0-80.5)	(13.3-66.5)				
MCP-1/CCL2[a]	54.1	71.8	55.3	90.2	**0.039**	1.000	1.000	0.018
	(23.4-145.8)	(17.4-146.3)	(31.9-148.3)	(71.5-151.2)				

MIF[a]	2769.2	2157.3	4888.6	7954.1	0.165	
	(385.7-20671.6)	(569.8-10415.0)	(383.8-2694463.0)	(198.2-15893269.0)		
TARC/CCL17[a]	165.9	287.9	303.6	279.0	0.417	
	(19.6-685.2)	(24.8-908.4)	(79.0-1837.3)	(67.6-1502.3)		
MIP-1α/CCL3[a]	27.1	25.8	21.0	57.1	0.490	
	(2.6-346.8)	(2.6-487.0)	(2.6-420.2)	(10.1-889.4)		
IL-26[a]	41.7	41.7	41.7	41.7	0.931	
	(41.7-1438.5)	(41.7-2065.6)	(41.7-3149.8)	(41.7-8422.2)		

[a]concentration of the mediator in pg/ml
[b]concentration of the mediator in ng/ml
[c]p-values derived from Kruskal-Wallis analysis
[d]p-values derived from post-Kruskal-Wallis Dunn's test
Serum of patients with different non-infectious uveitis entities as well as non-inflammatory controls was measured for different mediators. Data of 37 mediators are presented as median with their corresponding range. Significant p-values are highlighted in bold.
JIA-U = uveitis associated with juvenile idiopathic arthritis, CAU = chronic anterior uveitis without arthritis, IU = idiopathic uveitis, Controls = non-inflammatory controls, IL = interleukin, IFN = interferon, LAP = latency associated peptide, TGF = transforming growth factor, MIP = macrophage inflammatory protein, CCL = chemokine (C-C motif) ligand, MDC = macrophage derived cytokine, CXCL = chemokine (C-X-C motif) ligand, MIG = monokine induced by gamma interferon, BLC = B lymphocyte chemoattractant, OPG = osteoprotegerin, SAA = serum amyloid A, BDNF = brain-derived neurotrophic factor, VEGF = vascular endothelial growth factor, sICAM = soluble intracellular adhesion molecule, sVCAM = soluble vascular cell adhesion protein, CD = cluster of differentiation, sCD14 = soluble CD14, sIL-2R = soluble IL-2 receptor, Ang = angiopoietin, TIE = tyrosine kinase with immunoglobulin-like and EGF-like domains, TEK = tunica endothelial kinase, C5a = complement component 5a, MCP = monocyte chemoattractant protein, IP = interferon gamma-induced protein, MIF = macrophage migration inhibitory factor, ACE = angiotensin converting enzyme.

Chapter 5

Impact of uveitis on quality of life in adult patients with juvenile idiopathic arthritis

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ABSTRACT

Objective
To establish the impact of uveitis on the quality of life (QoL) in adult patients with juvenile idiopathic arthritis (JIA).

Methods
Adult patients with a history of JIA, both with (n = 31) or without (n = 51) chronic anterior uveitis, were included. Their scores on 3 validated QoL questionnaires (National Eye Institute Visual Functioning Questionnaire [NEI VFQ-25], Medical Outcomes Study 36-Item Short Form health survey [SF-36], and EuroQoL 5-domaine questionnaire [EQ-5D]) were analyzed to find factors that could influence QoL.

Results
The median overall composite score (OCS) of the NEI VFQ-25 was significantly worse in the uveitis group compared to the non-uveitis group (respectively, 83.4 [range 15.2-94.7] and 94.9 [range 46.3-100]; $P < 0.001$). Nearly all subscale scores were lower in patients with uveitis than in patients without uveitis ($P < 0.001$ for all). After adjusting for duration of arthritis, JIA subtype, arthritis onset before or after 1990, and the use of systemic immunomodulatory medication, the QoL was still worse in patients with uveitis (NEI VFQ-25 OCS regression coefficient = -11.7; $P = 0.002$). No significant differences were found between the groups for the SF-36 and the EQ-5D. In the total JIA group, the use of systemic medication appeared to negatively influence some general QoL scores.

Conclusion
Having a history of uveitis has a substantial negative effect on the vision-related QoL in JIA in adulthood, despite good visual acuity. General QoL scores did not differ between uveitis and non-uveitis patients, but the use of systemic immunomodulatory treatment, independent of uveitis, did negatively influence general QoL scores in adult JIA patients.

Significance & Innovations

- Having a history of uveitis has a substantial negative effect on the vision-related quality of life in adults with juvenile idiopathic uveitis, despite good visual acuity according to the Standardization of Uveitis Nomenclature and World Health Organization criteria.
- The general quality-of-life scores in adults with juvenile idiopathic arthritis do not differ between patients with or without a history of uveitis.
- Use of systemic immunomodulatory treatment negatively influences general quality-of-life scores in adult patients with juvenile idiopathic arthritis

INTRODUCTION

Chronic anterior uveitis is the most common extraarticular manifestation in patients with juvenile idiopathic arthritis (JIA), occurring in 11.6% to 30% of the patients, especially in those with an oligoarticular or polyarticular rheumatoid factor-negative subtype.[1-3] JIA is also the most common cause of uveitis in children.[3] Serious complications such as glaucoma, cystoid macular edema and hypotony might occur, sometimes even resulting in blindness.[4-6] A previous study found that 19% of the patients with childhood uveitis had at least 1 legally blind eye.[7] Reported percentages of blind eyes and visual loss in relation to uveitis in adulthood range from 5% to 20% in the western world.[8] The effect of vision loss on the quality of life (QoL) is one of the main concerns of patients with eye disease.[9] Even though JIA uveitis has its typical onset in childhood, clinical observations show that many patients can have active uveitis or are dealing with its complications and intensive treatment during adulthood as well. In 3 previous studies, almost half of the patients still had active uveitis with serious complications or were treated for ocular manifestations of JIA in adulthood.[10-12]

Previous research on the QoL in patients with uveitis alone and JIA alone showed a lower QoL in comparison to healthy subjects.[13-15] Recently, a cohort study in children only, on the QoL and function of JIA patients, showed that children with uveitis had poorer vision-related QoL and function compared to patients with JIA without uveitis.[16] So far, we lack information on the QoL in patients with JIA and uveitis in adulthood. The main purpose of our study is to establish the QoL of patients with JIA and a history of uveitis, compared to patients with JIA without a history of uveitis, and to identify factors that influence QoL in adulthood.

PATIENTS AND METHODS

Patients

A total of 194 adult patients with a history of JIA, with or without chronic anterior uveitis, who visited the ophthalmology department and/or rheumatology department of the University Medical Center of Utrecht, were approached in August 2014 to participate in this study. All patients received a patient information letter and an informed consent form. Additionally, they were sent 3 validated questionnaires: the National Eye Institute Visual Functioning Questionnaire (NEI VFQ-25), the Medical Outcomes Study 36-Item Short Form health survey (SF-36), and the EuroQoL 5-domaine questionnaire (EQ-5D). The Dutch validated versions of all these questionnaires were used. Also, an additional questionnaire with questions about patients' medical history was added. Patients were asked to complete the questionnaires as soon as possible after being approached and had the option to complete the questionnaires online or on paper at home. Patients were given this choice in order to increase the chance that every single patient would be able to read the questionnaires and answer them. A reminder was sent after 2 months if a patient had not responded.

Patients were included in the study if they were ages ≥18 years. Patients whose JIA was oligoarticular persistent, oligoarticular extended, or polyarticular rheumatoid factor-negative were included, since these JIA subtypes are specifically associated with silent chronic anterior uveitis, the most common form of JIA uveitis. For analyses, the oligoarticular extended

subtype was grouped together with the polyarticular subtype. The diagnosis of JIA was confirmed by a pediatric rheumatologist according to the criteria of the International League of Associations for Rheumatology, or by former criteria such as the criteria of the European League Against Rheumatism.[17,18] After 1993, uveitis was diagnosed during screening according to the recommendations of the American Academy of Pediatrics by an ophthalmologist, or patients were referred with uveitis, and JIA was diagnosed subsequently.[19,20] Uveitis was defined by the Standardization of Uveitis Nomenclature (SUN) international working group, and before 2005 according to the criteria at that time (Bloch-Michel and Nussenblatt, 1987).[21,22] Only patients with chronic anterior uveitis were included, and patients with recurrent acute uveitis were excluded from this study. Patients were divided into 2 groups: patients with JIA with a history of uveitis and those without. Some JIA patients (n = 4) were never screened by an ophthalmologist and never had any visual complaints. These patients were included in the JIA without uveitis group. Patients were excluded from this study if they had significant comorbidity unrelated to JIA or uveitis (e.g., diabetes mellitus, inflammatory bowel disease), major developmental disorders (e.g., mental disorder), and other systemic diseases associated with uveitis. This case-control study was approved by the Medical Ethics Committee of the University Medical Center of Utrecht and is in compliance with Helsinki principles. A written informed consent was obtained from all participating patients.

Data collection

In addition to the questionnaires, data were collected from patients' medical charts and included date of birth, sex, date of JIA onset, JIA subtype, laboratory records, and the use of systemic immunomodulatory treatment (IMT). Around 1990, with the advent of methotrexate and later biologic agents, the use of systemic IMT drastically changed and increased. Therefore, patients were subdivided according to the year of JIA onset, before or after the year 1990, for subanalyses. Uveitis-related data were collected from JIA uveitis patients including date of uveitis onset, ocular surgeries in the medical history, and unilateral or bilateral involvement. Also data on uveitis activity and ocular complications at least 6 months prior to completion of the questionnaires were collected. Active uveitis was defined as ≥0.5 positive cells in the anterior chamber and/or the presence of cystoid macular edema.[21] The most recent visual fields and best-corrected visual acuities (BCVAs) not older than 8 months were collected from the uveitis patients when available. The BCVAs were classified into 3 groups according to the visual acuity threshold of the SUN working group: no visual impairment, visual impairment (BCVA of 20/50 or worse), and legal blindness (BCVA of 20/200 or worse).[21] If patients had a visual field of 10˚ or less, they were also classified in the legal blindness group, according to the criteria of the World Health Organization (WHO).[23]

QoL and function measures

The NEI VFQ-25 is a measure for the vision-related QoL.[24] This validated self-administered questionnaire was developed by the RAND Corporation under the sponsorship of the National Eye Institute, to assess the impact of visual impairment on QoL. It consists of 25 questions, with a total score ranging from 0 to 100 (where 0 = the lowest vision-related QoL and 100 = the highest vision-related QoL). It includes 11 vision-related domains (general vision, near vision, distance vision, driving, peripheral vision, color vision, ocular pain, vision-specific role difficulties, vision-specific dependency, vision-specific social function, and vision-specific mental health) and 1 general health item. The overall composite score (OCS)

includes the 11 vision-related domains, excluding the general health item. The SF-36 is a General Health Survey that is composed of 36 questions and standardized response choices, organized into 8 multi-item scales.[25,26] These scales are physical functioning, social functioning, role limitations due to physical health problems, role limitations due to emotional problems, general mental health, vitality, bodily pain, and general health perceptions. Subscale scores range from 0 to 100, with higher scores indicating better QoL.

The EQ-5D is a standardized health-related QoL questionnaire.[27,28] It consists of items on 5 dimensions: mobility, self-care, daily activities, pain/discomfort, anxiety/depression, and a visual analog scale (VAS) from 0 to 100. Each dimension has 3 ranks: no problems, some problems, and severe problems. The numbers for 5 dimensions can be combined in a 5-digit number describing the respondent's health state, which then is converted to an EQ index ranging from -0.33 to 1 (where -0.33 = worst health-related and 1 = best health related QoL).[29]

The additional questionnaire included 19 questions about medical history, information on performed surgeries, and use of systemic IMT at the time of completion of the questionnaires. It took our patients approximately 30 minutes to fill out all 4 questionnaires.

Statistical analysis

Data were analyzed using IBM SPSS statistics software, version 21. The completed questionnaires were imported in NetQuestionnaires before they were exported to SPSS. Statistical significance was at *P* values less than 0.05 level. Correction for multiple comparisons was done using false discovery rate (FDR). Both nominal *P* values and FDR-corrected statistical significance were reported. We compared baseline demographic and clinical variables of adults with JIA without a history of uveitis to adults with JIA with a history of uveitis using chi-square tests or Fisher's exact test. The Mann-Whitney U test was used for the comparison of nonparametric data. We performed initial descriptive analyses of clinical variables of adults with a history of JIA uveitis. Data were summarized using the median with range and mean. Factors that were significantly different between the 2 groups in univariable analysis and could possibly influence the QoL outcome were selected for a multivariable linear regression analysis. Correlations between these factors were first analyzed by the Spearman's correlation test. If 2 factors were strongly and significantly correlated, only 1 of them was selected for multivariable analysis. For subanalyses, only patients with known data on that subject were included for the specific analyses (n values are reported).

RESULTS

Demographics and disease characteristics

A total of 82 patients of the 194 who received questionnaires responded. Statistically, more women responded than men (63 of 128 women [49%] and 19 of 66 men [29%]; *P* = 0.006). There were no differences in occurrence of uveitis (31 of 67 patients [46%] with a history of uveitis responded, and 51 of 127 patients [40%] without a history of uveitis or an unknown uveitis status responded; *P* = 0.413). There were also no differences in JIA subtype or age of arthritis or uveitis onset between the responding and nonresponding group. Of the 82 patients who responded, 31 had JIA with uveitis, and 51 had JIA without uveitis. **Table 1**

summarizes the characteristics of included subjects. The uveitis characteristics of included subjects are described in **Table 2**.

Table 1. Characteristics of adults with juvenile idiopathic arthritis (JIA) with and without uveitis*

Characteristics	Total (n=82)	JIA (n=51)	JIA uveitis (n=31)	*P*
Sex				
Women	64 (78)	40 (78)	24 (77)	0.915†
Men	18 (22)	11 (22)	7 (23)	-
Age, median (range) years	22.3 (18.1-66.9)	20.9 (18.3-31.1)	28.6 (18.1-66.9)	0.002‡
Age arthritis onset, median (range) years	7.2 (0.7-21.1)	9.2 (0.7-15.8)	3.7 (0.7-21.1)	0.016‡
Age uveitis onset, median (range) years	NA	NA	5.2 (1.8-43.7)	NA
Duration arthritis, median (range) years	16.2 (1.3-59.2)	14.4 (4.1-25.7)	23.3 (1.3-59.2)	<0.001‡
Duration uveitis, median (range) years	NA	NA	19.6 (2.7-59.1)	NA
JIA subtype				
Oligoarticular	34 (41)	18 (35)	16 (64)	0.018§
Polyarticular¶	42 (52)	33 (65)	9 (36)	-
ANA				
Positive	49/76 (64)	28/50 (56)	21/26 (81)	0.032§
HLA-B27				-
Positive	8/30 (27)	4/16 (25)	4/14 (29)	0.825†
Systemic medication** (n=81)#				-
Yes	53 (65)	29 (57)	24 (80)	0.034§
No	28 (35)	22 (43)	6 (20)	-
Joint surgery				
Yes	27 (33)	14 (28)	13 (42)	0.176†
No	55 (67)	37 (72)	18 (58)	-
Arthritis onset decade				
Before 1990	15 (18)	1 (2)	14 (45)	<0.001§
In or after 1990	67 (82)	50 (98)	17 (55)	-

* Values are the number (%) unless indicated otherwise. NA = not applicable; ANA = antinuclear antibodies.
† Chi-square test.
‡ Statistically significant using the Mann-Whitney U test.
§ Statistically significant using the chi-square test.
¶ Patients who were diagnosed with oligoarticular extended JIA were grouped in the polyarticular subgroup.
# Number of patients with known data.
** No medication: no systemic medication or only use of nonsteroidal antiinflammatory drugs.
Immunosuppressants: oral corticosteroids, methotrexate, azathioprine, or other disease-modifying antirheumatic drugs (leflunomide, mycophenolate motefil, or cyclosporine), or anti-tumor necrosis factor (etanercept, infliximab, or adalimumab) and/or other biological agents (tocilizumab, rituximab, or abatacept).

Table 2. Uveitis characteristics of patients with juvenile idiopathic arthritis (JIA)-associated uveitis*

Characteristics	JIA uveitis (n=31)
Onset of disease with	
Arthritis	25 (81)
Uveitis	6 (19)
Uveitis diagnosis decade	
Before 1990	11 (35)
In or after 1990	20 (65)
Uveitis laterality	
Unilateral	4 (13)
Bilateral	27 (87)
Visual acuity†	
No. of patients	23
Bilateral	
No visual impairment	19 (82)
Visual impairment	2 (9)
Legal blindness	2 (9)
Unilateral	
No visual impairment	16 (69)
Visual impairment	2 (9)
Legal blindness	5 (22)
Eye surgery	
No. of patients	31
Ocular surgery in medical history	25 (81)
Cataract	22 (71)
Glaucoma	13 (42)
Uveitis activity at last visit‡	
No. of patients	23
Active	11 (48)

*Values are the number (%) unless indicated otherwise.
† Defined by the Standardization of Uveitis Nomenclature international working group and World Health Organization, measured no longer than 8 months before filling out the questionnaire.
‡ Duration between date of completing the questionnaires and last visit is no longer than 6 months. Uveitis activity is defined as ≥0.5 positive cells in the anterior chamber and/or having cystoid macular edema.

Vision-related QoL

The NEI VFQ-25 scores are shown in **Table 3**. Nearly all subscale scores as well as the OCS were lower in JIA patients with a history of uveitis than in patients with JIA without a history of uveitis ($P < 0.001$), with the exception of general health and driving ($P = 0.397$ and $P = 0.187$, respectively). These scores were comparable in both groups. To examine whether these differences in scores were only influenced by extremes, we did an additional analysis where we compared patients with a history of uveitis and patients without a history of uveitis, after exclusion of patients with bilateral visual impairment or legal blindness (n = 4) from the uveitis group; the results on all NEI VFQ-25 subscales remained comparable with the previously mentioned results (all but general health and driving; P < 0.001).

Table 3. National Eye Institute Visual Function Questionnaire (NEI VFQ-25) subscale scores and overall composite score*

	Total (n=81)			JIA (n=50)			JIA uveitis (n=31)			
NEI VFQ-25 subscales	**Median**	**(range)**	**Mean**	**Median**	**(range)**	**Mean**	**Median**	**(range)**	**Mean**	**Nominal *P*†**
General health (n=79/50/29)	50.0	(0.0-100.0)	54.8	50.0	(0.0-100.0)	56.5	50.0	(0.0-100.0)	51.7	0.397
General Vision (n=79/50/29)	80.0	(0.0-100.0)	78.2	80.0	(40.0-100.0)	86.0	80.0	(0.0-100.0)	64.8	<0.001‡
Ocular pain	87.5	(0.0-100.0)	83.0	100.0	(37.5-100.0)	92.5	62.5	(0.0-100.0)	67.7	<0.001‡
Near vision	100.0	(8.3-100.0)	88.8	100.0	(58.3-100.0)	98.0	83.3	(8.3-100.0)	73.9	<0.001‡
Distance vision (n=80/50/30)‡	91.7	(8.3-100.0)	83.7	100.0	(33.3-100.0)	92.2	79.2	(8.3-100.0)	69.6	<0.001‡
Social functioning	100.0	(25.0-100.0)	92.4	100.0	(75.0-100.0)	99.5	100.0	(25.0-100.0)	81.0	<0.001‡
Mental health	93.8	(18.8-100.0)	86.0	93.8	(43.8-100.0)	93.7	81.3	(18.8-100.0)	73.7	<0.001‡
Role difficulties	100.0	(0.0-100.0)	85.3	100.0	(37.5-100.0)	96.3	75.0	(0.0-100.0)	67.7	<0.001‡
Dependency	100.0	(25.0-100.0)	93.9	100.0	(41.7-100.0)	98.7	100.0	(25.0-100.0)	86.2	<0.001‡
Driving (n=54/34/20)	58.7	(0.0-67.3)	51.8	58.7	(17.8-67.3)	55.5	54.5	(0.0-67.0)	45.3	0.187
Color vision	100.0	(25.0-100.0)	96.6	100.0	(100.0-100.0)	100.0	100.0	(25.0-100.0)	91.1	<0.001‡
Peripheral vision	100.0	(25.0-100.0)	88.3	100.0	(46.3-100.0)	97.0	75.0	(25.0-100.0)	74.2	<0.001‡
Overall composite score	91.8	(15.2-100.0)	85.3	94.9	(46.3-100.0)	92.9	83.4	(15.2-94.7)	73.0	<0.001‡

* (n=…/…/…) indicates the number of patients responding on the item, when there are differences from the number shown at the top of the column. JIA = juvenile idiopathic arthritis.
† By Mann Whitney-U-test.
‡ Significant nominal p-values, and significant false discovery rate-corrected P values.

General health and QoL

The results of the SF-36 and the EQ-5D are shown in **Supplementary Tables 1 and 2**. There were no significant differences between the JIA and JIA uveitis group for the SF-36 scores, nor for the EQ-5D index or VAS score.

Multivariable analysis of possible risk factors affecting the QoL. To analyze whether the differences in NEI VFQ-25 outcomes were a direct consequence of having a history of uveitis, multivariable analysis was performed. Significant correlations were found between duration of arthritis and age at the time of completion of the questionnaires (r_s = 0.730, P < 0.001), between duration of arthritis and duration of uveitis (r_s = 0.726, P < 0.001) and between duration of arthritis and age of JIA onset (r_s = -0.709, P < 0.001). Therefore, duration of arthritis was entered into multivariable analysis. In addition, the JIA subtype, current use of systemic IMT, and arthritis onset before or after the year 1990 were entered into multivariable analysis. When adjusted for duration of arthritis, JIA subtype, use of systemic IMT, and arthritis onset before or after 1990, having a history of uveitis was statistically significantly associated with a lower score on various NEI VFQ-25 subscales (Supplementary **Table 3**). Many subscale scores and the OCS were also negatively influenced by an extended duration of JIA. When adjusted for a history of uveitis, duration of arthritis, JIA subtype, and arthritis onset before or after 1990, the use of systemic IMT at the time of completion of the questionnaires appeared to negatively influence the outcomes of the general health score of the NEI-VFQ-25, as well as some scores of the SF-36 and the EQ-5D VAS score (**Supplementary Table 3**). Outcomes (significant *P* values and directions of the regression coefficients [B] in **Supplementary Table 3**) were comparable for most of the subscale scores after replacing duration of arthritis with age at the time of completing the questionnaires in the before mentioned multivariable analysis, except for general vision, ocular pain (NEI-VFQ-25), and physical functioning (SF-36). These scores were only significantly negatively influenced by the presence of a history of uveitis and not by a higher age (data not shown).

Analyses within the JIA uveitis group

Of the 23 patients with JIA uveitis from whom data on visual acuity was available, 4 (17%) had bilateral visual impairment or were blind, and 7 patients (30%) had at least one visual impaired or blind eye. Patients with unilateral or bilateral visual impairment or blindness scored statistically significantly lower on 9 of the 13 subscales of the NEI VFQ-25 (**Supplementary Table 4**). These visually impaired or blind patients did not score lower on SF-36 items or EQ-5D scores compared to JIA uveitis patients with normal visual acuity (data not shown).

When comparing patients with known activity status, patients with active uveitis appeared to score lower on 2 subscales of the NEI VFQ-25 (driving and ocular pain), compared to patients with quiescence of their uveitis (**Supplementary Table 5**). Patients with bilateral uveitis scored lower on 2 NEI VFQ-25 subscales, compared to patients with unilateral uveitis. Patients who had eye surgery (for cataract and/or glaucoma) in their medical history scored lower on 4 visual functioning questionnaire subscale scores and on 1 SF-36 score, compared to patients without eye surgeries.

Patients with uveitis onset before arthritis did not score differently from patients with arthritis onset before uveitis on any of the questionnaires.

DISCUSSION

Our study shows that the vision-related QoL is significantly lower in adults with JIA with a history of uveitis compared to those with JIA without a history of uveitis. To our knowledge, this is the first study that describes the impact of uveitis on the QoL in adult JIA patients. Multivariable analysis confirmed our univariable analysis findings that the presence of uveitis, as well as extended duration of JIA, uveitis, or higher age at the time of completing the questionnaires are important independent risk factors for a decreased vision-related QoL. In one study, a higher age was already known to be related to decreased QoL scores. Nevertheless, in our study, after correction for age, having a history of uveitis also independently affected some QoL scores.[30] Although as expected, QoL scores were lower for visually impaired or blind JIA uveitis patients compared to patients with no visual impairment, JIA uveitis patients with a good visual acuity scored lower than JIA patients without uveitis on NEI VFQ-25 items. This result might indicate that the presence of a history of uveitis in the absence of visual impairment, according to the SUN or WHO criteria, does not guarantee a good vision-related QoL.[21,23]

We found statistically significant lower NEI VFQ-25 scores on most subscales for JIA uveitis compared to non-uveitis patients (**Table 3**). The question arises as to whether these statistical differences are also of clinical relevance. We lack research on clinically relevant scores for JIA and JIA uveitis, but the Age-Related Eye Disease Study (AREDS) investigated the responsiveness (defined as "the ability of a measurement tool to detect meaningful change in populations over time" or "sensitivity to change") of the NEI VFQ-25 scores in age-related macular degeneration.[31] The researchers found changes in NEI VFQ-25 scores of 10 points or more to be associated with clinically significant changes. In our study, all mean differences, except for color vision, were higher than 10 points by comparison, suggesting clinical relevant differences between the groups (rather than change within a group as in the AREDS study) (**Table 3**). Color vision did show a statistically significant difference in uveitis versus non-uveitis patients, but this difference was <10 points. In addition, the score for color vision in JIA uveitis patients was still >90 points, which might not be clinically relevant.

Previously, Angeles-Han et al did a similar comparison on the vision-related QoL in JIA patients during childhood with and without uveitis and confirmed that the uveitis group had a worse vision-specific QoL.[16] They used the Effects of Youngsters Eyesight on Quality of Life to measure vision-related QoL and function. This questionnaire for children is comparable with the NEI VFQ-25 for adults. Angeles-Han et al found a statistically significantly lower score (3.35 on a 4.00-point scale) for patients with uveitis compared to patients without uveitis (3.68 on a 4.00-point scale; $P < 0.001$). If we compare our JIA uveitis NEI VFQ-25 subscale scores to the NEI VFQ-25 scores of healthy subjects in the study of Mangione et al and Hirneiss et al, our patients show lower scores. Hirneiss et al reported a mean NEI VFQ-25 OCS of 91.6, compared to our score of 73.0 (the OCS score of the JIA patients without uveitis was 92.9 in our group, and thus comparable to healthy subjects).[24,30]

Several studies investigated the QoL in other uveitis entities. Schiffman et al studied adults with different types of uveitis by using the NEI VFQ-25 and found a lower QoL in uveitis patients compared to healthy subjects.[14] Their uveitis patients scored lower than ours; the median age of uveitis patients in our study was lower (28.6 years) compared to the patients in the study of Schiffman (42.3 years). Furthermore, only 17% of the patients from that study had anterior uveitis, whereas the uveitis patients in our study had typical JIA-associated chronic anterior uveitis. These factors might be explanations for the difference on the NEI

VFQ-25 subscales.[14] Hoeksema and Los performed a QoL study on patients with herpetic anterior uveitis and found a NEI VFQ-25 OCS of 88.1 and a NEI VFQ-25 general health score of 59.0, which are both higher than in our JIA uveitis group, but lower than the scores found in healthy subjects.[13,30] The mean patient's age in Hoeksema and Los's study was higher (57.7 years) than in our study (28.6 years). In a previous study, a higher age was found to negatively influence the NEI VFQ-25 outcomes.[30] These results together imply that uveitis in general has a serious influence on the vision-related QoL, but the specific chronic anterior uveitis associated with JIA might affect the vision-related QoL even more seriously. In our series there was no significant difference for the SF-36 and EQ-5D between the 2 groups. So general and health-related QoL do not seem to be influenced by the presence of uveitis. However, compared to the healthy population in a previous study, the SF-36 scale scores were lower in our complete cohort, both for the group with and the group without uveitis.[25,26,32] The SF-36 General Health mean score in this healthy population was 71.9 compared to 53.3 in JIA and 56.4 in JIA uveitis; the SF-36 mental health mean score was 74.7 in this healthy population, and in our study group it was 58.7 in JIA and 60.1 in JIA uveitis.[26] This indicates the impact of suffering from JIA in young adult life. These results are in concordance with Foster et al who did a specific study on QoL in adults with JIA (the presence of uveitis was not described in that study) compared to healthy controls, using the SF-36; JIA patients had significantly lower SF-scores on 6 of the 8 items.[15]

In our study, the use of systemic IMT at the time of completion of the questionnaires significantly influenced NEI VFQ-25 scores for general health, as well as part of SF-36 scores and the EQ-5D VAS score. This finding is interesting, as we recently described an increase in the use of systemic IMT for JIA and uveitis since the year 1990.[12] Therefore, these outcomes indicate the need for new treatment options and personalized care for patients with JIA and JIA uveitis. In future studies, it will also be interesting to examine which specific medical treatments influence the QoL of patients, which was not possible in our cohort due to heterogeneity of treatment. Furthermore, it will be useful to examine whether these treatments were indicated for patients' arthritis or uveitis. In any case, this investigation will be a challenge, because systemic IMTs are frequently started for both indications, arthritis as well as uveitis.

Despite the fact that JIA uveitis typically is an asymptomatic disease, ocular pain scores were worse in patients with active uveitis compared to those with no active uveitis. In the NEI VFQ-25 the questions concerning ocular pain enclose a broad spectrum of pain, namely burning, itching, or aching. Topical treatment of active uveitis accompanied by corneal irritation might explain the lower pain score in patients with activity.

Four JIA patients were never screened by an ophthalmologist, so we included them in the non-uveitis group since they had never had any visual complaints and had never been treated for uveitis, making it plausible that they indeed indeed did not have a history of uveitis. To be certain whether these 4 patients significantly influenced our outcomes, we also performed univariable and multivariable analyses after excluding these 4 patients, and our outcomes and conclusions remained the same (data not shown). Based on the NEI VFQ-25 scoring algorithm from Mangione et al, we conclude that our sample sizes were large enough to detect differences in the 2 groups, except for the subscale for driving, which might explain why we did not find a significant difference for this subscale.[24] EuroQoL advises a sample size of at least 44 per group in the EQ-5D, which might explain why we did not find significant differences between the 2 groups on the EQ-5D scores.[33] Furthermore, the questionnaires were all completed at a different moment in the disease process, though

this was the case in both groups, so such confounding could be prevented by stratification. This study was conducted at the University Medical Center of Utrecht, a tertiary referral center, thus our study groups may not accurately represent the general JIA population. We tried to prevent this problem by sending questionnaires to patients that were treated in the past in our center, but not at the moment of this study. The interval between last ophthalmologic examination and filling out the questionnaires (for activity and BCVA) was at least 6-8 months, which was quite extended, but JIA uveitis is a chronic disease, which might fluctuate all the time. Additionally, there is the possibility of selection bias for patients with poor visual acuity, which we tried to prevent as much as possible by giving patients the opportunity to fill out the questionnaires either online or on paper. We assumed an increase in response rate with this approach. For future QoL studies, we would advise to measure QoL at the moment of visit at the outpatient department, which makes it possible to link clinical outcomes to QoL outcomes and also to decrease possible selection bias.
In conclusion, our study shows that the vision-related QoL is lower in adults with JIA with a history of uveitis compared to those with JIA without a history of uveitis, despite good visual acuity. General QoL scores (SF-36 and EQ-5D) did not differ between uveitis and non-uveitis patients. But the use of systemic IMT influenced the general QoL of JIA patients, independent from having a history of uveitis. It is important for ophthalmologists and rheumatologists to realize the impact of this disease on a patient's QoL when treating adults with JIA and JIA uveitis.

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SUPPLEMENTARY TABLES

Supplementary Table 1. Medical Outcomes Study 36-Item Short Form (SF-36) Subscale scores

	Total (n=82)			JIA (n=51)			JIA-uveitis (n=31)			
SF-36 Subscales	**Median**	**(range)**	**Mean**	**Median**	**(range)**	**Mean**	**Median**	**(range)**	**Mean**	**P-value[a]**
Physical Functioning	82.5	(0.0-100.0)	75.9	85.0	(30.0-100.0)	78.5	75.0	(0.0-100.0)	71.8	0.696
Role-Physical	75.0	(0.0-100.0)	64.3	100.0	(0.0-100.0)	67.6	75.0	(0.0-100.0)	58.9	0.338
Bodily Pain	46.0	(22.0-88.0)	50.0	50.0	(22.0-84.0)	50.8	42.0	(31.0-88.0)	48.8	0.823
General Health (n=81/51/30)[b]	57.0	(25.0-82.0)	54.5	57.0	(30.0-82.0)	53.3	57.0	(25.0-77.0)	56.4	0.251
Vitality (n=81/51/30)[b]	50.0	(15.0-75.0)	47.4	50.0	(15.0-75.0)	47.1	50.0	(30.0-70.0)	48.0	0.714
Social Functioning	50.0	(0.0-62.5)	44.2	50.0	(12.5-62.5)	44.9	50.0	(0.0-50.0)	43.1	0.535
Role-Emotional	100.0	(0.0-100.0)	80.5	100.0	(0.0-100.0)	83.0	100.0	(0.0-100.0)	76.3	0.297
Mental Health (n=81/51/30)[b]	60.0	(40.0-76.0)	59.4	60.0	(40.0-76.0)	58.7	60.0	(44.0-76.0)	60.1	0.153

JIA: juvenile idiopathic arthritis. JIA-uveitis: JIA associated uveitis. [a]Mann Whitney-U-test was used. [b](n=.../.../...) indicates the number of patients responding on the item, if not specified, the number corresponds with what is shown at the top of the table.

Supplementary Table 2. EuroQol group EQ-5D

	Total (n=81)			JIA (n=50)			JIA-uveitis (n=31)			
EQ-5D items	**Median**	**(range)**	**Mean**	**Median**	**(range)**	**Mean**	**Median**	**(range)**	**Mean**	**P-value[a]**
EQ-5D Index	0.8	(0.1-1.0)	0.8	0.8	(0.1-1.0)	0.8	0.8	(0.1-1.0)	0.8	0.581
EQ-5D VAS scale	74.0	(2.0-100.0)	71.1	72.5	(2.0-100.0)	70.3	74.9	(30.5-100.0)	72.4	0.834

JIA: juvenile idiopathic arthritis. JIA-uveitis: JIA associated uveitis. [a]Mann Whitney-U-test was used.

Supplementary Table 3. Outcomes of multivariable lineair regression analysis of risk factors for quality of life scores

	Adjusted R^2	Intercept	Uveitis (yes/no)			Duration arthritis			JIA subtype			Systemic IMT			Arthritis onset (</≥1990)		
NEI-VFQ-25			*B*	*95%-CI*	*p-value*	*B*	*95%-CI*	*p-value*	*B*	*95%-CI*	*p-value*	*B*	*95%-CI*	*p-value*	*B*	*95%-CI*	*p-value*
General Health (n=79)	0.276	40.28	10.75	(0.09, -1.89)	.094	-0.06	(-0.60, 0.49)	.839	3.44	(-6.84. 13.72)	.506	-15.77	(-26.20, -5.34)	**.004***	27.32	(14.14, 40.50)	**.000***
General Vision (n=79)	0.238	87.24	-14.78	(-26.53, -3.04)	**.014**	-0.57	(-1.07, -0.06)	**.028**	5.23	(-4.32. 14.78)	.278	1.75	(-7.94, 11.44)	.719	4.23	(-8.01, 16.47)	.493
Ocular Pain	0.401	99.08	-17.94	(-26.44, -9.44)	**.000***	-0.39	(-0.76, -0.03)	**.037**	-1.37	(-8.36. 5.62)	.697	-3.58	(-10.67, 3.52)	.318	1.57	(-7.37, 10.51)	.727
Near Vision	0.429	104.26	-11.80	(-20.59, -3.02)	**.009***	-0.79	(-1.17, -0.41)	**.000***	3.56	(-3.67. 10.78)	.330	-1.47	(-8.80, 5.86)	.690	4.74	(-4.50, 13.98)	.310
Distance Vision (n=80)	0.330	94.16	-12.49	(-23.41, -1.57)	**.026**	-0.63	(-1.09, -0.17)	**.008***	7.17	(-1.72. 16.06)	.112	-3.82	(-12.71, 5.07)	.394	7.07	(-4.28, 18.42)	.218
Social Functioning	0.417	101.00	-6.43	(-13.55, 0.68)	.076	-0.66	(-0.97, -0.35)	**.000***	1.96	(-3.9. 7.81)	.507	0.89	(-5.05, 6.82)	.767	7.26	(-0.23, 14.75)	.057
Mental Health	0.219	99.24	-15.41	(-24.47, -6.35)	**.001***	-0.30	(-0.69, 0.09)	.134	1.53	(-5.92. 8.98)	.683	-0.62	(-8.18, 6.94)	.870	-1.84	(-11.37, 7.70)	.702
Role Difficulties	0.267	94.57	-19.74	(-31.82, -7.65)	**.002***	-0.41	(-0.94, 0.11)	.120	3.01	(-6.93. 12.96)	.547	1.16	(-8.93, 11.24)	.819	6.36	(-6.36, 19.07)	.322
Dependency	0.074	99.74	-8.13	(-16.24, -.02)	**.049**	-0.19	(-0.54, 0.16)	.278	2.81	(-3.85. 9.48)	.403	0.19	(-6.57, 6.96)	.955	0.54	(-7.99, 9.08)	.899
Driving (n= 54)	0.108	45.64	-2.63	(-13.24, 7.98)	**.620**	-0.19	(-0.69, 0.31)	.451	3.57	(-5.96. 13.09)	.454	-0.50	(-9.37, 8.36)	.909	13.53	(2.24, 24.82)	.020
Color Vision	0.230	102.54	-1.12	(-5.15, 2.90)	**.579**	-0.32	(-0.49, -0.14)	**.001***	-0.98	(-4.28. 2.33)	.558	0.62	(-2.73, 3.98)	.712	2.09	(-2.15, 6.32)	.329
Peripheral Vision	0.279	104.89	-12.62	(-23.90, -1.34)	**.029**	-0.65	(-1.14, -0.16)	**.010***	1.18	(-8.1. 10.46)	.801	-4.77	(-14.18, 4.64)	.316	3.83	(-8.04, 15.70)	.522
Overall Composite Score	0.387	95.32	-11.68	(-18.85, -4.52)	**.002***	-0.48	(-0.79, -0.17)	**.003***	2.91	(-2.98. 8.81)	.328	-0.50	(-6.48, 5.48)	.867	3.90	(-3.64, 11.44)	.306

SF-36			*B*	*95%-CI*	*p-value*	*B*	*95%-CI*	*p-value*	*B*	*95%-CI*	*p-value*	*B*	*95%-CI*	*p-value*	*B*	*95%-CI*	*p-value*
Physical Functioning	0.239	84.52	7.41	(-5.18, 20.00)	.244	-0.65	(-1.20, -0.11)	**.020**	8.40	(-1.95, 18.74)	.110	-14.29	(-24.76, -3.81)	**.008**	9.15	(-4.10, 22.40)	.173
Role-Physical	0.078	5.71	0.23	(-0.69, 1.15)	.620	0.02	(-0.02, 0.06)	.360	0.59	(-0.16, 1.35)	.121	-0.64	(-1.40, 0.13)	.102	0.98	(0.01, 1.95)	**.047**
Bodily Pain	0.042	60.48	3.61	(-6.85, 14.07)	.494	-0.15	(-0.61, 0.30)	.507	-5.57	(-14.17, 3.03)	.200	-11.30	(-20.00, -2.59)	**.012**	0.95	(-10.07, 11.96)	.864
General Health	0.045	54.34	0.99	(-6.40, 8.38)	.790	0.06	(-0.25, 0.38)	.693	-4.56	(-10.59, 1.48)	.136	4.40	(-1.70, 10.50)	.155	-3.09	(-10.85, 4.67)	.430
Vitality	-0.050	44.71	1.17	(-5.73, 8.07)	.737	-0.04	(-0.33, 0.26)	.798	-1.11	(-6.74, 4.53)	.696	0.01	(-5.68, 5.71)	.996	3.62	(-3.63, 10.87)	.322
Social Functioning	-0.036	48.59	-0.11	(-7.10, 6.89)	.976	-0.10	(-0.40, 0.21)	.522	-2.41	(-8.16, 3.34)	.406	-2.66	(-8.48, 3.17)	.366	0.00	(-7.37, 7.36)	1.000
Role-Emotional	-0.030	79.10	-0.19	(-21.62, 21.24)	.986	0.39	(-0.54, 1.32)	.404	-0.05	(-17.66, 17.56)	.995	-13.01	(-30.84, 4.82)	.150	6.48	(-16.08, 29.05)	.568
Mental Health	0.000	59.53	3.53	(-0.85, 7.91)	.112	0.00	(-0.19, 0.19)	.988	-2.20	(-5.77, 1.38)	.225	-3.05	(-6.66, 0.57)	.097	1.89	(-2.71, 6.48)	.416
EQ-5D			*B*	*95%-CI*	*p-value*	*B*	*95%-CI*	*p-value*	*B*	*95%-CI*	*p-value*	*B*	*95%-CI*	*p-value*	*B*	*95%-CI*	*p-value*
EQ-5D Index	0.200	0.71	0.08	(-0.03, 0.20)	.163	0.00	(-0.01, 0.00)	.181	0.06	(-0.04, 0.16)	.237	-0.09	(-0.19, 0.01)	.069	0.18	(0.06, 0.31)	**.005***
EQ-5D VAS scale	0.056	67.38	9.60	(-3.55, 22.74)	.150	0.14	(-0.44, 0.72)	.641	1.12	(-9.74, 11.99)	.837	-13.55	(-24.62, -2.49)	**.017**	9.19	(-5.39, 23.77)	.213

B = Regression coefficient; CI = confidence interval; IMT = immunomodulatory treatment; '(n=...)'= when not all 82 patients filled in this specific item, the total number of patients is reported here.The outcomes of the multivariable analysis on the outcome scores of the three quality of life questionnaires are shown. These outcomes were derived from lineair regression analysis. For the different categorical variables, dummy variables were used as follows, for uveitis: Yes=1, no=0; JIA subtype: Oligoarticular JIA=1, polyarticular or extended oligoarticular JIA=0; for systemic IMT: Useage of IMT=1; no IMT=1; for arhtritis onset: In or after the year 1990=1, before the year 1990=0. An example: The score of ocular pain was statistically significant influenced by uveitis and duration of arthritis after adjusting for JIA subtype, use of systemic IMT and arthritis onset before or in/after the year 1990; having uveitis decreased the score and the longer the arthritis lasted, the lower the score. Significant nominal p-values are highlighted in bold. *Significant FDR-corrected p-values.

Supplementary Table 4. Comparison within JIA-uveitis patients, stratifying for bilateral visual acuity

	No visual impairment[a] (n=19)			Visual impaired/Blind[a] (n=4)			
NEI VFQ-25 Scores	**Median**	**(range)**	**Mean**	**Median**	**(range)**	**Mean**	**Nominal P-value**
General Health (n=18/4)[b]	50.0	(0.0-100.0)	52.8	50.0	(25.0-50.0)	43.8	0.538
General Vision (n=18/4)[b]	80.0	(40.0-100.0)	73.3	40.0	(20.0-60.0)	40.0	**0.014***
Ocular Pain	75.0	(50.0-100.0)	74.3	56.3	(50.0-62.5)	56.3	**0.044**
Near Vision	91.7	(33.3-100.0)	85.1	33.3	(16.7-66.7)	37.5	**0.003***
Distance Vision (n=18/4)[b]	83.3	(37.5-100.0)	80.3	20.8	(8.3-58.0)	27.1	**0.001***
Social Functioning	100.0	(62.5-100.0)	93.4	50.0	(25.0-75.0)	50.0	**0.002***
Mental Health	81.3	(18.8-100.0)	77.0	78.1	(75.0-93.8)	81.3	0.969
Role Difficulties	75.0	(25.0-100.0)	76.3	50.0	(12.5-62.5)	43.8	**0.021***
Dependency	100.0	(33.3-100.0)	94.3	79.2	(58.3-91.7)	77.1	**0.009***
Driving (n=14/0)[b,c]	58.7	(17.3-67.0)	52.2		NA		NA
Color Vision	100.0	(75.0-100.0)	98.7	87.5	(50.0-100.0)	81.3	0.162
Peripheral Vision	100.0	(25.0-100.0)	86.8	37.5	(25.0-50.0)	37.5	**0.003***
Overall Composite Score	87.1	(42.5-94.6)	81.8	49.5	(43.7-70.0)	53.2	**0.006***

Analyses on the NEI VFQ-25 scores were performed within the JIA-uveitis patients of whom visual status was known. [a]Visual acuity was defined by the Standardization of Uveitis Nomenclature (SUN) international working group and World Health Organization (WHO), measured no longer than 8 months before filling in the questionnaire. [b](n=.../...) indicates the number of patients responding on the item, if not specified, the number corresponds with what is shown at the top of the table. [c]All 9 patients who did not finish the questions on the driving item had never driven a car; they did not stop because of their visual acuity. Significant nominal p-values are highlighted in bold. *Significant FDR-corrected p-values.

Supplementary Table 5. Statistical significant differences for patients with JIA-uveitis, stratified for different characteristics

	Ever surgery (n=25)			Never surgery (n=6)			
NEI VFQ-25 Eye surgery[a]	**Median**	**(range)**	**Mean**	**Median**	**(range)**	**Mean**	**Nominal P-value**
General Vision (n=23/6)[b]	60.0	(0.0-100.0)	60.0	80.0	(60.0-100.0)	83.3	**0.032**
Near Vision	75.0	(8.0-100.0)	68.0	100.0	(91.7-100.0)	98.6	**0.011**
Dependency	91.7	(25.0-100.0)	83.0	100.0	(100.0-100.0)	100.0	**0.029**
Overall Composite Score	74.6	(15.2-94.6)	68.9	90.3	(83.4-94.7)	89.9	**0.024**
SF-36-Eye surgery							
Changes in health perception	50.0	(0.0-50.0)	43.0	62.5	(50.0-75.0)	62.5	**0.004**

	Unilateral (n=4)			Bilateral (n=27)			
NEI VFQ-25-Laterality uveitis	**Median**	**(range)**	**Mean**	**Median**	**(range)**	**Mean**	**Nominal P-value**
Mental Health	96.9	(81.3-100.0)	93.8	81.3	(18.8-93.8)	70.7	**0.019**
Role Difficulties	100.0	(75.0-100.0)	93.8	62.5	(0.0-100.0)	63.9	**0.045**

	No activity (n=12)			Activity (n=11)			
NEI VFQ-25-Uveitis activity	**Median**	**(range)**	**Mean**	**Median**	**(range)**	**Mean**	**Nominal P-value**
Ocular Pain	75.0	(50.0-100.0)	79.2	62.5	(50.0-87.5)	62.5	**0.017**
Driving (n=7/7)[b]	67.0	(42.3-67.0)	61.1	50.3	(17.3-58.7)	43.3	**0.016**

Analyses on all questionnaire's subscale scores were performed within the JIA-uveitis patientgroup (n=31), stratifying for different factors. Here the results of the factors which were statistically significant different from eachother are presented. None of the nominal p-values remained statistically significant after FDR-correction. Uveitis activity is defined as having at least 0.5+ cells and/or cystoid macular edema in the last 6 months before filling in the questionaires. [a]Ten patients had cataract surgery only, 1 patient had glaucoma surgery only, 12 patients had both cataract and glaucoma surgery. Patients were aged 3.5-53.4 years when having their cataract surgery and 8.6-43.7 years when having their glaucoma surgery; [b](n=.../...) indicates the number of patients responding on the item, if not specified, the number corresponds with what is shown at the top of the table.

Chapter 6

Impact of juvenile idiopathic arthritis associated uveitis in early adulthood

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ABSTRACT

Background
Typically juvenile idiopathic arthritis (JIA)-associated uveitis (further referred as 'JIA-uveitis') has its onset in childhood, but some patients suffer its, sometimes visual threatening, complications or ongoing disease activity in adulthood. The objective of this study was to analyze uveitis activity, complications and visual prognosis in adulthood.

Methods
In this multicenter study, 67 adult patients (129 affected eyes) with JIA-uveitis were retrospectively studied for best corrected visual acuity, visual fields, uveitis activity, topical/systemic treatments, ocular complications, and ocular surgeries during their 18th, 22nd and 30th year of life. Because treatment strategies changed after the year 1990, outcomes were stratified for onset of uveitis before and after 1990.

Results
Sixty-two of all 67 included patients (93%) had bilateral uveitis. During their 18th life year, 4/52 patients (8%) had complete remission, 28/52 (54%) had uveitis activity and 37/51 patients (73%) were on systemic immunomodulatory treatment. Bilateral visual impairment or legal blindness occurred in 2/51 patients (4%); unilateral visual impairment or legal blindness occurred in 17/51 patients (33%) aged 18 years. The visual prognosis appeared to be slightly better for patients with uveitis onset after the year 1990 (for uveitis onset before 1990 (n=7) four patients (58%) and for uveitis onset after 1990 (n=44) 30 patients (30%) were either visual impaired or blind). At least one ocular surgery was performed in 10/24 patients (42%) between their 18th and 22nd year of life.

Conclusions
Bilateral visual outcome in early adulthood in patients with JIA-uveitis appears to be fairly good, although one third of the patients developed one visually impaired or blind eye. However, a fair amount of the patients suffered from ongoing uveitis activity and needed ongoing treatment as well as surgical interventions. Awareness of these findings is important for ophthalmologists and rheumatologists treating patients with JIA-uveitis, as well as for the patients themselves.

INTRODUCTION

Chronic anterior uveitis is the most common extra-articular manifestation of juvenile idiopathic arthritis (JIA), a serious disease starting prior to the age of 16 years.[1] During the course of, or preceding JIA, uveitis occurs in 10% up to 45% of the patients, depending on the specific subtype of JIA.[1-3] Typically JIA-associated uveitis (further referred as 'JIA-uveitis') has its onset in childhood, but some patients suffer its, sometimes visual threatening, complications or ongoing disease activity in adulthood. Common complications include band keratopathy, posterior synechiae, cataract, secondary glaucoma and cystoid macular edema (CME).[4-6] However, previous clinical studies seldom investigated a follow-up of more than seven years.[7-11] Previously, we reported on uveitis activity in childhood and puberty and this study revealed a flare up of uveitis during puberty.[12] Little is known about JIA-uveitis activity in adulthood, but there are suggestions that uveitis activity may persist in adulthood in 30-63% of the patients. Reported outcomes differ between various studies due to broad follow-up ranges.[12-18] Therefore, the course of uveitis activity and outcome in adulthood remain unclear. Furthermore, treatment strategies changed drastically with the upcoming of methotrexate (MTX) around 1990 and later anti-TNFα therapy around the year 2000.[19-22] Also, ophthalmologic screening protocols for patients with JIA were introduced around 1990.[23-25] The objective of this study was to analyze uveitis activity and its complications and visual prognosis in adults with JIA-uveitis and to compare patients with an onset of uveitis before and after the year 1990.

METHODS

A retrospective chart review of 67 patients with JIA-associated chronic anterior uveitis who had reached the age of 18 years, examined at the tertiary centers University Medical Center of Utrecht, Rotterdam, Leiden and Groningen during the period of 1974 to 2015, was performed. The majority of the included patients were of European descent. In 12 patients, follow-up data at the tertiary centers were not complete, therefore these patients signed an informed consent form in order to make a copy of the ophthalmologist's patient's medical chart from general hospitals, where these patients had also been treated. These charts were additionally reviewed. This study was approved by the Institutional Review Board of the Utrecht University Medical Center and is in compliance with Helsinki principles. The Review Board agreed that patients' informed consent was not necessary, since the data were analyzed anonymously.

JIA was diagnosed and classified by a pediatric rheumatologist according to the criteria of the International League of Associations for Rheumatology (ILAR) or by former criteria, such as the European League Against Rheumatism (EULAR).[26,27] The uveitis diagnosis was made by an ophthalmologist according to the recommendations of the International Uveitis study group.[28]

The following demographic and disease characteristics were documented for every patient: date of birth, gender, JIA subtype, laterality of the uveitis, age at onset of arthritis, age at onset of uveitis, uveitis onset before 1990 or after 1990, the presence of anti-nuclear antibodies (ANA) and the presence of Human Leukocyte Antigen B-27 (HLA-B27). Furthermore, the occurrence of synechiae and band keratopathy and patients' age at onset of these complications were noted, as well as the cataract and glaucoma surgeries being

performed, and the age at which these were done. Finally, the subsequent data were collected at fixed time-points, namely during the year in which the patients became 18, 22 and 30 years old: activity of uveitis, use of topical corticosteroids, use of intraocular pressure (IOP) lowering medication, use of systemic immunomodulatory treatment (IMT), occurrence of complications like CME, papillitis, and hypotony (defined as an IOP <6mmHg in at least two consecutive visits), best corrected visual acuity (BCVA) measured with Snellen charts and visual field defects based on examination with the Rodenstock Peritest or the Humphrey Field Analyzer. When BCVA was measured more times during a year, the best BCVA of the age year was used for analyses.

Patients diagnosed with uveitis after the age of 16 years and patients with a JIA subtype other than juvenile oligoarthritis, extended oligoarthritis or polyarthritis (rheumatoid factor negative) were excluded. Uveitis was considered active when there were at least 1+ cells in the anterior chamber, as determined by the grading system of the Standardization of Uveitis Nomenclature (SUN) working group, at least at one visit during the year of the fixed time-point.[29] Remission was defined as inactive disease for ≥ three months after discontinuing all treatments for eye disease (SUN). Systemic IMT was noted when used for more than six consecutive months and included MTX, corticosteroids, adalimumab, infliximab, other disease modifying anti-rheumatic drugs (mycophenolate mofetil, azathioprine or cyclosporine), other biologicals (tocilizumab or etanercept) or other anti-rheumatic drugs (hydroxychloroquine or sulfasalazine). Starting dose of MTX was 10-15 mg/m^2 body surface once a week, with a maximum of 20 mg/m^2, and starting dose of oral prednisone was generally 1mg/kg body weight. If compliance with the usage of medication was reported in the patient's medical chart, these data were used. Changes in medication, uveitis activity and complications arising within a period of two months after surgery were not included. Measurements of visual acuity were only included for analyses if uninfluenced by or corrected for refractive errors (BCVA).

The database was built per patient, with additionally included information per eye for BCVA, visual field and ocular surgeries. Visual outcome was classified into three groups, based on the criteria of the SUN working group: visual acuity better than 20/50 was defined as no visual impairment, visual acuity equal to or less than 20/50 was defined as visual impairment and visual acuity equal to or less than 20/200 was defined as legal blindness.[29] If patients had a visual field of 10˚ or less they were also classified as 'legal blindness', according to the criteria for visual impairment of the World Health Organization.[30] Data on visual ability (visual acuity in combination with visual field outcome) per eye were combined to analyze the visual impairment per patient, primarily using data of the best eye to determine the visual outcome. If data of the worst eye was used, this is explicitly mentioned.

To study the course of the BCVA, Snellen visual acuity values were transferred to LogMar. When patients had a visual field of ≤10°, the same LogMar visual acuity as for light perception was used (LogMar = 2.90). For patients who had no light perception we used a LogMar visual acuity of 3.20. The reported BCVA's in this manuscript are reported on a Snellen scale and are based on per eye analyses.

Statistical analysis was performed using SPSS 21.0 for Windows (IBM Corporation, Armonk, New York, USA). Normality was tested using the Shapiro-Wilk test. Medians combined with a range were given for not normally distributed variables. A Chi-squared test or Fisher's exact test was performed to compare categorical data and a Mann-Whitney U-test was done to compare medians across groups. To analyze the course of the disease in patients of whom data was available at 18 and 22 years of age and at 18, 22 and 30 years, the McNemar test

was used to compare dichotomous variables. To compare the visual acuity in these patients, Snellen BCVA was log transformed and median BCVA's (with interquartile range (IQR)) were compared by the Wilcoxon signed rank test.

RESULTS

General characteristics of study population

Sixty-seven patients in total were included (129 affected eyes) and 62 (93%) had bilateral uveitis. Characteristics of the study population at the age of 18, 22 and 30 years (n= 52, 26 and 13 respectively) with uveitis onset before 1990 and after 1990 are shown in **Table 1**. All data at 30 years were from patients with uveitis onset before 1990. There were no statistically significant differences for baseline characteristics after stratifying for uveitis onset before and after the year 1990.

Visual impairment and visual acuity

Data of the visual ability at the different ages are shown in **Figure 1**. At the age of 18 years, 2/51 patients (4%) were visually impaired or legally blind. One was diagnosed before 1990 and one after 1990. Seventeen out of 51 patients (33%) had at least one eye which had a visual acuity of 20/50 or worse and/or a visual field of ≤10° of which 13/17 patients were diagnosed after 1990 (which represents 30% of all 44 patients diagnosed after 1990) and 4/17 patients were diagnosed before 1990 (which represents 58% of all seven patients diagnosed before 1990). Of all patients with an impaired visual ability of at least one eye, 10 out of 17 (59%) were diagnosed with uveitis before arthritis versus five out of 33 (15%) of the patients with a good visual ability (P = .001). Also at the age of 22 years, the two patients (100%) with visual impairment of the best eye were diagnosed with uveitis before arthritis, compared to three out of 22 patients (14%) with normal visual acuity (P = .036).

No differences were found for gender, uveitis onset before 1990 or after 1990, or total number of ocular surgeries since uveitis onset.

The course of visual acuity could be measured for 23 patients with follow-up data at both 18 and 22 years. The median BCVA between 18 and 22 years (n=23 patients) appeared to be stable. Sixteen of these patients (70%) were diagnosed after 1990. Six patients had follow-up data at all age years (18, 22 and 30 years) and had a median BCVA of one eye of 20/63 (IQR 20/20-0.25/200) at the age of 18 years and 2/200 (IQR 20/22-0.25/200) at the age of 30 years (P = .465) and for the other eye a median BCVA of 20/25 at 18 years (IQR 20/16-20/100) and 20/40 at 30 years (IQR 20/25-0.02/200; P = .116). All these six patients were diagnosed before the year 1990.

Table 1. Characteristics of patients with juvenile idiopathic arthritis associated uveitis at the age of 18, 22 and 30 years

			< 1990			≥ 1990		
Characteristics		**Total**	**18 years**	**22 years**	**30 years**	**18 years**	**22 years**	**30 years**
Total no. patients		67	7	8	13	45	18	0
Total no. eyes		129	14	16	26	86	36	NA
Bilateral uveitis no. patients (%)		62 (93)	7 (100)	8 (100)	13 (100)	41 (91)	18 (100)	NA
Female, no. patients (%)		50 (75)	6 (86)	7 (88)	12 (92)	30 (67)	13 (72)	NA
Median age (y) at onset uveitis (range)		5.2 (1.2-14.6)	4.1 (3.0-9.3)	4.5 (3.0-9.3)	4.9 (3.0-9.3)	5.3 (2.6-14.6)	5.2 (2.6-12.7)	NA
Median age (y) at onset arthritis (range)		3.5 (0.8-16.1)	3.0 (0.8-7.4)	2.9 (0.8-7.4)	3.0 (0.8-7.4)	3.8 (0.9-16.1)	4.7 (1.0-12.7)	NA
Interval (y) arthritis-uveitis, median (range)		0.5 (-9.8-13.3)	0.7 (-3.4-6.5)	1.6 (-3.4-6.5)	0.7 (-3.4-6.5)	0.4 (-9.8-13.3)	0.5 (-6.7-6.0)	NA
Median duration (y) of uveitis (range)		NA	13.9 (8.7-15.0)	17.5 (12.7-19.0)	25.1 (20.7-27.0)	12.7 (3.4-15.4)	16.9 (9.3-19.4)	NA
Arthritis onset before uveitis, no. patients (%)		48 (74)	5 (83)	6 (86)	9 (82)	30 (67)	13 (72)	NA
JIA subtype, no. patients (%)	Oligoarthritis	35 (52)	4 (57)	4 (50)	5 (38)	28 (62)	9 (50)	NA
	Extended oligoarthritis	9 (13)	0 (0)	0 (0)	1 (8)	7 (16)	5 (28)	NA
	Polyarthritis	15 (22)	1 (14)	1 (13)	2 (16)	8 (18)	4 (22)	NA
	Unknown	8 (12)	2 (29)	3 (37)	5 (38)	2 (4)	0 (0)	NA
ANA, no. patients	Total	64	7	8	12	44	18	NA
	Positive (%)	52 (81)	6 (86)	7 (88)	11 (92)	37 (84)	15 (83)	NA
HLA-B27, no. Patients	Total	28	1	2	5	18	8	NA
	Positive (%)	5 (18)	0 (0)	0 (0)	1 (20)	3 (17)	0 (0)	NA

JIA = juvenile idiopathic arthritis; ANA = antinuclear antibodies; HLA-B27 = human leukocyte antigen B27; NA = not applicable. Per age-group statistical analyses were performed comparing patients with an uveitis diagnosis before (<) or after (≥) 1990 by the Fisher exact test and Mann-Whitney U test. No statistically significant differences were found. In the group with an uveitis diagnosis <1990, more data were available at age 30 years than at age 18 or 22 years. Because of the retrospective character of the study, more data were missing at the latter two time-points.

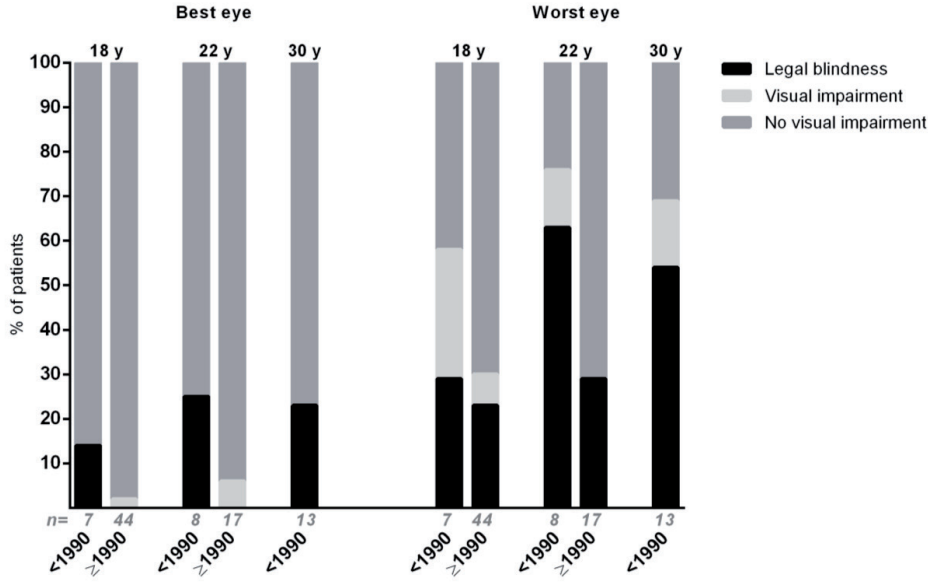

Figure 1. *Visual impairment of patients with juvenile idiopathic arthritis associated uveitis. Proportion of patients with juvenile idiopathic arthritis associated uveitis with no visual impairment, visual impairment or legal blindness of the best (left) and worst (right) eye at the age of 18, 22 and 30 years, with uveitis onset before (<) 1990/after (≥) 1990.*
Visual acuity better than 20/50 was defined as no visual impairment, visual acuity equal to or less than 20/50 was defined as visual impairment and visual acuity equal to or less than 20/200 was defined as legal blindness.[29] *If patients had a visual field of 10˚ or less they were also classified as 'legal blindness', according to the criteria for visual impairment of the World Health Organization.*[30] *Statistical analyses comparing the situation <1990 and ≥1990 were derived from the Fisher exact test, there were no statistically significant differences. 'n=' = total number of patients included in the bar.*

Uveitis activity and treatment

Of the 52 patients with available data at the age of 18 years, four patients (8%) were in remission during the entire year (**Figure 2**). Almost half of the patients (n=28/52, 54%) had an episode of active uveitis during their 18th life year, 37/51 patients (73%) were on systemic IMT and 42/52 (81%) used topical steroids. Four of the 37 (11%) 18 year-old patients on systemic IMT had no uveitis activity and did not use topical steroids (all diagnosed ≥1990). All others in the group 'without remission' were at least on topical corticosteroids or had uveitis activity.

The majority of 18 year-old patients diagnosed after 1990 were on systemic IMT (n=36/45, 80%), in contrast to the patients diagnosed before 1990, where only 1/6 patients (17%) was on systemic IMT during the 18th life year (*P* = .004). A comparable result was found at the age of 22 years (*P* = .017, **Figure 2**). No other statistically significant differences were found for uveitis activity or treatment between the patients diagnosed before and after 1990.

The different treatment strategies are presented in **Figure 3**. The figure shows that patients diagnosed after 1990 were treated with biologicals more often than patients diagnosed with uveitis before 1990.

Course of complications and surgery

Ten of the 24 patients (42%) with follow-up data available at the age of 18 as well as at the age of 22 years underwent surgery between their 18th and 22nd year of age. Three/24 patients (14%) underwent cataract surgery and 9/24 (38%) underwent glaucoma surgery between their 18th and 22nd birthday.

Seventeen/24 patients (71%) had already had surgery by the time they became 18 years old (6/24 patients had had surgery for cataract and glaucoma, 10/24 for cataract only, 1/24 for glaucoma only). Four/24 patients (17%) had their first surgery after the age of 18. Three/24 patients (13%) never underwent surgery before their 22nd life year. Note that all of these 24 patients had bilateral uveitis and the majority of patients (n=17/24, 71%) was diagnosed with uveitis after 1990.

Figure 4 presents the number of cataract surgeries and type of glaucoma surgeries performed in the complete study population. Though not statistically significant, these results suggest that patients underwent glaucoma surgery more often when having an uveitis onset after 1990.

None of the patients developed posterior synechiae between the age of 18 and 22 years, though one patient (4%) developed band keratopathy.

Active inflammation in the anterior chamber was less frequent at the 22nd life year (8/23, 35%) compared to the 18th life year (15/23, 65%; $P = .039$). No differences were found for complete remission of uveitis, presence of CME, use of topical steroids, use of systemic IMT, use of IOP lowering medication, papillitis or hypotony.

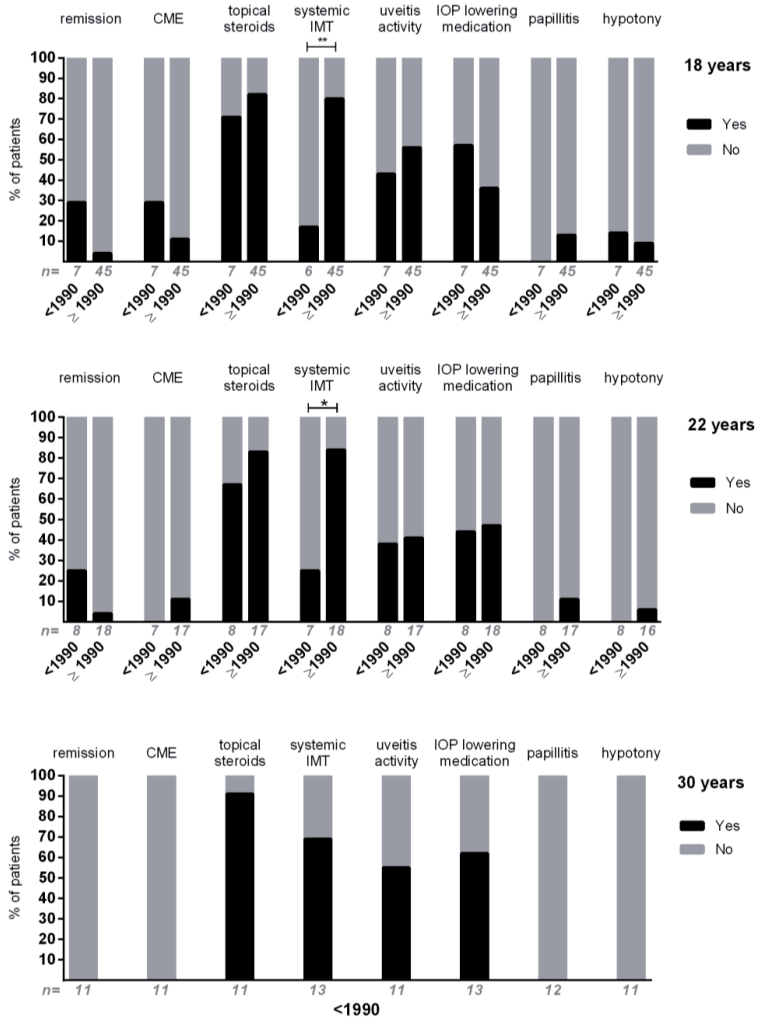

Figure 2. *Uveitis activity, treatment and complications of patients with uveitis associated with juvenile idiopathic arthritis in adulthood. Outcomes of patients with uveitis onset before (<) and after (≥) the year 1990. At the top: outcomes at age 18; middle: age 22 years; bottom: age 30 years. Note that all data at age 30 years were from patients with uveitis onset before the year 1990.*

Remission was defined as inactive disease for ≥ three months after discontinuing all treatments for eye disease.[29] *Systemic immunomodulatory treatment (IMT) was defined 'Yes' when used for more than six months. Uveitis activity was defined 'Yes' when there were at least 1+ cells in the anterior chamber, as determined by the grading system of the Standardization of Uveitis Nomenclature (SUN) working group.*[29] *CME = cystoid macular edema; IOP = intraocular pressure. *: P < .05; **: P < .005. 'n=' = total number of patients included in the bar.*

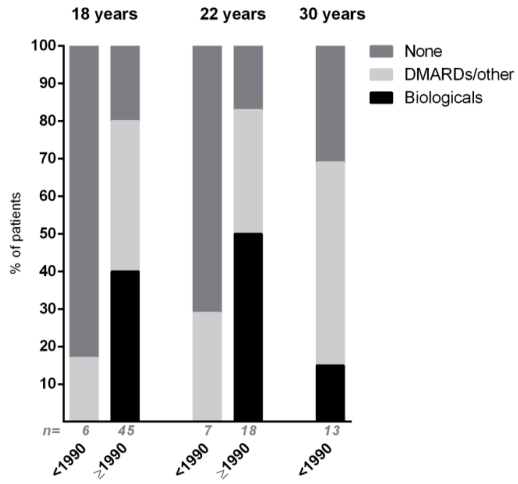

Figure 3. *The use of systemic immunomodulatory treatment in patients with juvenile idiopathic arthritis associated uveitis. Treatment at the age of 18, 22 and 30 years with uveitis onset before (<) and after (≥) the year 1990. DMARD = disease modifying anti-rheumatic drugs. A biological was always given combined with a DMARD (in the figure noted as 'Biologicals'), a DMARD/other drugs were usually given as combination therapy. 'n=' = total number of patients included in the bar. The exact treatment, with number of patients using this treatment in brackets, was: Treatment 18 years, <1990: Corticosteroids (n=1). Treatment 18 years, ≥ 1990: Corticosteroids (n=1), Methotrexate (n=31), Mycophenolate motefil (n=2), Azathioprine (n=2), Cyclosporine (n=1), Adalimumab (n=16), Etanercept (n=1), Infliximab (n=2). Treatment 22 years, < 1990: Corticosteroids (n=1), Methotrexate (n=1). Treatment 22 years, ≥ 1990: Corticosteroids (n=2), Methotrexate (n=9), Mycophenolate motefil (n=3), Azathioprine (n=1), Hydroxychloroquine (n=2), Adalimumab (n=4), Etanercept (n=2), Tocilizumab (n=2), Infliximab (n=1). Treatment 30 years, <1990: Corticosteroids (n=4), Methotrexate (n=5), Mycophenolate motefil (n=1), Hydroxychloroquine (n=1), Cyclosporine (n=1),* Sulfasalazine *(n=1), Tocilizumab (n=1), Etanercept (n=1).*

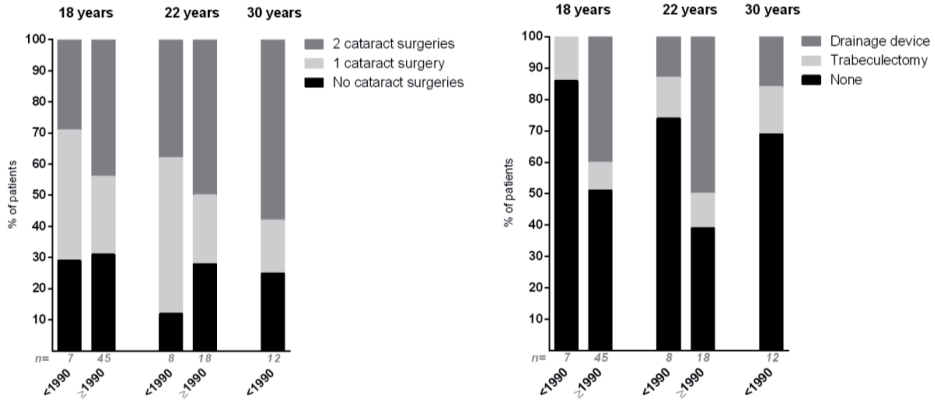

Figure 4. *Cataract and glaucoma surgery of patients with uveitis associated with juvenile idiopathic arthritis in adulthood. Proportion of patients who had undergone cataract (left) and glaucoma (right) surgery by the time they were 18, 22 and 30 years old, with uveitis onset before (<) and after (≥) the year 1990. Many patients had multiple glaucoma surgeries; in this figure patients with at least one glaucoma surgery versus no glaucoma surgeries are presented. Some of the patients who received a drainage device, also had a trabeculectomy in their medical history and are here included in the 'Drainage device' group. There were no statistically significant differences between the groups with uveitis onset before and after 1990. 'n=' = total number of patients included in the bar.*

DISCUSSION

Our results document that JIA is not solely a disease of childhood, but that its activity, accompanied use of systemic medications, complications and additional visual loss continues in early adulthood. Only 4% of the JIA-uveitis patients were in remission at 18 years of age.

Previous literature, as presented in **Table 2**, described patients with JIA-uveitis in adulthood, but broad ranges of follow-up make it difficult to interpret their results. In our study we studied different outcome measurements at fixed time-points. Further, to get a good impression of the visual ability of the patients, visual acuity cannot be seen apart from visual field outcomes. Only one previous study of 12 adult JIA-uveitis patients also studied visual field outcomes.[14]

Our study showed a trend towards a slightly better visual outcome for patients diagnosed after 1990 than for patients diagnosed before 1990. Also, a higher percentage of patients diagnosed after 1990 used systemic IMT, which suggests an improvement of visual outcome due to novel and more intensive immunomodulating treatment strategies since 1990.[19-22] It is likely that the use of ophthalmologic screening guidelines for patients with JIA has also contributed to improved visual outcome.[23-25] Furthermore, the awareness of glaucoma increased during the last decades with earlier and improved interventions and more prevention of severe glaucomatous visual field loss, as also displayed by **Figure 4**.[31,32] Also, cataract extractions are nowadays performed at an earlier stage, preventing development of amblyopia. Previous literature described a delay of cataract formation in patients treated with MTX, which means that, since the introduction of MTX as a treatment for JIA-uveitis, children have less chance to develop cataract at an age vulnerable for developing amblyopia.[33]

Better visual outcome in relation to systemic treatment was also shown by the study of Gregory et al.[4] Unfortunately, we could not correlate use of systemic IMT to visual outcome, since our study had data at three time-points rather than continuous data over the years. Additionally, the prescription of IMT might have been influenced by uveitis as well as arthritis activity. Anyhow, only four patients were solely on IMT without uveitis activity or topical steroids for their uveitis, suggesting that most patients in this study received systemic IMT at least partially for their uveitis.

Though our results suggest that most patients with JIA-uveitis have a fairly good binocular visual prognosis, with a rather stable visual outcome during the adolescent years, about one third of the patients with uveitis onset after the year 1990 had at least one visually impaired eye (**Figure 1**). In line with previous literature, risk factors for an impaired visual outcome were onset of uveitis before arthritis and lower age at onset of uveitis.[34] Because of the relatively small sample size in our patient groups, we cannot exclude that other factors which were described previously, such as male gender, are also possible risk factors for visual impairment.[6]

Despite the slight improvement of visual acuity and the increased use of systemic IMT after 1990, uveitis in early adulthood was active in approximately half of the patients. This is an important finding to be aware of, since a previous study described uveitis activity to be associated with an increased risk of visual loss.[4] It is extremely important for ophthalmologists to keep a close eye on these patients and treat them accurately, to prevent them from more bi- or unilateral visual impairment due to uveitis activity in their future lives. A longer follow-up and a larger cohort are necessary to be able to document their visual functioning over the years.

Our study cohort presents a representative JIA-uveitis population, as the male-to-female ratio, median age of arthritis onset and presence of ANA are similar to previous literature.[12,14-17,35,36]

Because of its retrospective character our study has limitations. There might have been a tertiary referral bias, since patients were selected from a cohort of tertiary centers. Though, this was minimized as much as possible by retrieving medical charts from patients being followed also by their own ophthalmologists outside the tertiary centers. Therefore, the percentage of patients with active uveitis and/or complications during adulthood might be lower than presented in this study. The sample size of patients evaluated at the age of 30 years is also limited, and moreover, the data of these patients at their 18th and 22nd year of life were not available, because it was not possible to retrieve their old medical charts.

Overall results on visual outcomes in this study are quite promising, with an improvement in visual acuity, probably also due to systemic IMT. Several studies have shown that that the introduction of MTX and anti-TNFα therapies have been important in the prevention of complications or even the occurrence of uveitis in JIA patients.[4,37] But these treatment strategies might have significant impact on the quality of life of patients. For example, use of systemic IMT will result in more visits to the ophthalmologists or rheumatologists, also, these young adult women might wish to become pregnant which conflicts with the use of most immunosuppressive medications. For future research it would be interesting to investigate the effect of the use of systemic IMT on these aspects, including quality of life of these adult patients.

In conclusion, the results of this study imply that binocular visual outcome in adulthood is fairly good in most patients with JIA-uveitis and that binocular visual ability seems to be stable in the first years after puberty. Despite the apparent improvement in visual prognosis in the last decades, still up to 30% of all adult JIA-uveitis patients have developed severe visual impairment or even blindness of at least one eye. Also, uveitis activity is continuing in early adulthood, and the majority of patients need ongoing treatment. Additionally, uveitis associated complications still arise in early adulthood and a relevant proportion of the patients need cataract or glaucoma surgery during adulthood. Awareness of these findings is important for ophthalmologists and rheumatologists treating patients with JIA-uveitis, as well as for the patients themselves, in order to prevent these patients from becoming visually disabled even many years after their typical childhood onset of this disease.

Table 2. Juvenile idiopathic arthritis associated uveitis in adulthood, a literature overview

Author	Nr patients	Mean/median age years (range)	Follow-up	Visual acuity	Definition active uveitis	Uveitis activity	Complications, treatment
Packham et al 2002[13]	54	35 (19-78) (= age of total JIA group with 246 patients)	Situation at the end of follow-up.	NA	Not defined	NA	66% of the patients had glaucoma during follow-up, 55% had cataract, 69% had had eye-surgery (not specifically during adulthood).
Zak et al 2003[14]	12	32 (22-49)	Situation at the end of follow-up.	Studied visual field outcomes.[a] 1 patient with bilateral severe visual field loss. 1 patient with unilateral VA <0.02.	Not defined	NA	Almost all patients had anterior segment findings as sequelae.
Ozdal et al 2005[15]	18 (30 eyes)	30 (18-48)	Situation at the end of follow-up. Minimum follow-up = 2 years.	9 patients (50%) had a BCVA <20/150 of at least one eye.	Presence of cells or keratic precipitates, with or without flare.	19 (63%) of the eyes had active uveitis.	3 eyes with phtisis. 73% (22 eyes) had cataract extraction, 4 eyes (13%) had glaucoma surgery by drainage device or trabeculectomy (not specifically during adulthood). 11 patients (61.1%) required the use of a systemic immunosuppresive agent. 16/18 patients were on topical steroids.
Kotaniemi et al 2005[16]	19	24 (22-26)	One consult for evaluation.	100% binocular normal BCVA, 3 patients had unilateral BCVA <0.1.	Use of topical corticosteroids and/or at least 3 cells in the anterior chamber.	8 patients had active uveitis.	4 patients had glaucoma, 5 had cataract. 53% of the patients were on systemic IMT. 10/19 patients used treatment (systemic and/or topical).
Camuglia et al 2009[17]	17	30 (21-43)	Situation at the end of follow-up.	20% of the eyes had visual loss up to 6/12, 13.3% of the eyes had visual loss up to 6/60.	Not defined	NA	53% of the patients (9 patients, 13 eyes) had new complications of cataract or glaucoma after their 16th birthday. Two eyes had glaucoma surgery and 10 eyes had cataract surgery after the age of 16. 30% of the patients had synechiae during their uveitis course. 10/17 patients used systemic treatment, all patients had topical treatment.

Skarin et al 2009[35]	55	NA	Follow-up at 24 years (range 18-46) after uveitis onset.	NA	Not defined	49% of the 55 patients had signs of active uveitis or were receiving topical corticosteroids.	12 patients (33%) had glaucoma, 28 patients (78%) had cataract.
Oray et al 2016[18]	77 (135 eyes)	29.7 (±11)	Situation at the end of follow-up.	37 eyes (28%) had a visual acuity of ≤20/50. 20 eyes (15%) had a visual acuity of ≤20/200.	≥0.5+ cells in the anterior chamber.	78 eyes (58%).	Ocular surgery in 68 eyes. 13 patients (17%) were treated with conventional IMT (e.g. MTX), 52 patients (68%) were treated with biologicals. At least one complication in 95 eyes (72%).

This table describes all previous literature on the course of juvenile idiopathic arthritis associated uveitis in adulthood.
[a]This was the only study which described visual field outcomes.
NA = not applicable; IMT = immunomodulatory treatment; MTX = Methotrexate

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Chapter 7

Summary
Discussion and future perspectives

SUMMARY

In **chapter 1**, we outlined the disease characteristics, course, treatment and understanding of the pathophysiology of juvenile idiopathic arthritis (JIA) associated uveitis (further referred as '*JIA-associated uveitis*') according to the current state of knowledge. Up to one third of the patients with the most common categories (oligoarticular and polyarticular rheumatoid factor negative) JIA will develop uveitis, usually within 4 years after arthritis onset. Likewise, JIA is the most common systemic association in children with uveitis. Despite this strong association and the discovery of various clinical and molecular markers linked to uveitis, it remains challenging to predict the development of uveitis in advance. This is further complicated by the typical insidious disease course of the characteristic chronic anterior uveitis. This asymptomatic clinical 'prelude' is not noticed by affected children until irreversible damage has occurred and accordingly demands careful and intensive ophthalmological screening to detect uveitis at an early stage. In this chapter, we propose several unmet research needs to cover critical gaps in the understanding of JIA-associated uveitis; For example, JIA as well as non-infectious uveitis are considered to be driven by T helper-1 and T helper-17 cells, but the mechanisms (if any) that simultaneously link these cell subsets to inflammatory reactions to both joints and eyes remains poorly understood. Aqueous humour (AqH) provides an excellent tissue to study the molecular perturbations near the primary side of inflammation. Recent *genome-wide association studies* (GWAS) in the uveitis-prone categories of JIA or in cohorts of non-infectious uveitis patients have identified several risk loci significantly associated with these multifactorial diseases, thus, we postulate that the use of GWAS may enable detailed genetic interrogation of uveitis susceptibility in JIA. Finally, studies on the visual prognosis or the Q*uality of Life* (QoL) related to JIA-associated uveitis have been limited to investigations during childhood, but, evidently there is also need for studies on these important clinical parameters in patients with longstanding disease that have entered adulthood. Bearing in mind the need for clinical, genetic and molecular biomarkers to predict the occurrence of uveitis in JIA and to understand its disease mechanism, and the need for studies on clinical parameters and QoL in adulthood, in this thesis, we studied the ocular micro-environment, assessed the genetic susceptibility, and investigated clinical and laboratory parameters in patients with JIA-associated uveitis. In addition, we investigated the prognosis and QoL of young adults with JIA-associated uveitis.

Clinical predictors for uveitis in JIA

In **chapter 2** our main aim was to study inflammatory parameters at arthritis onset as possible predictors for the development of uveitis in JIA. Inflammatory parameters and demographic and clinical factors were studied in 147 JIA patients with and 211 JIA patients without uveitis. Erythrocyte Sedimentation Rate (ESR) was more elevated in the uveitis group compared to the non-uveitis group. We could also confirm the established risk factors for uveitis in JIA: lower age of JIA onset, and anti-nuclear antibody (ANA) positivity. To test the clinical relevance of the newly found predictor ESR, we designed a model, taking into account the already established predictors (ANA, age of arthritis onset, and JIA-subtype) and compared the uveitis risk in different situations (**Figure 1** in chapter 2).

In conclusion, we found increased ESR at JIA onset to be predictive for uveitis occurrence in JIA. ESR is already routinely tested in patients at JIA onset, so its use as a biomarker can easily be implemented in daily practice.

Genetic susceptibility in JIA-associated uveitis

In **chapter 3** we conducted a GWAS to compare the frequencies of s*ingle nucleotide polymorphisms* (SNPs) in 192 JIA patients with uveitis and 330 JIA patients without uveitis using extended ophthalmological follow-up data. Patients in two independent cohorts from the Netherlands, Germany, Belgium and Switzerland were genotyped followed by (*major histocompatibility complex,* genome-wide) SNP imputation and collectively investigated by mega-analysis. We identified the amino acid serine at position 11 (serine-11) encoded by the *human leukocyte antigen (HLA)-DRB1* gene associated to uveitis in JIA. The serine-11 amino acid resides in '*the YST-motif*' in the bottom of the peptide binding groove of *HLA-DR*. This motif is comprised of tyrosine (Y) at position 10, serine (S) at position 11, and threonine (T) at position 12. Consequently, all three amino acids were in perfect linkage disequilibrium and confer identical risk to the development of uveitis in JIA. To explore the biological relevance of the associated variation in the *HLA-DRB1* locus, we investigated whether the associated motif would influence the peptide binding preferences of *HLA-DR*. To this end, we *in silico* predicted the binding affinities of all 15-mer overlapping peptides from selected proteins found in iris tissues (n~150) to 13 common *HLA-DRβ1* allotypes. This revealed that the binding affinities of *HLA-DRB1* alleles that encode the YST-motif were distinct from alleles that did not encode this motif.

Intriguingly, the serine-11 signal in uveitis was found to be female specific. In fact, only 1 female uveitis patient (1%) did not carry the allele encoding serine-11 (**Table 1**). Strikingly, this female appeared to suffer from peripheral multifocal choroiditis, without anterior segment inflammation, thus did not suffer from the characteristic (chronic anterior) uveitis phenotype linked to oligoarticular JIA.

In conclusion we found the YST-motif in the *HLA-DRB1* gene to be a genetically distinct, female specific feature of JIA-associated uveitis compared to non-uveitis JIA. This association indicates potential involvement of antigen presentation by *HLA-DRβ1* in the development of uveitis in JIA.

Table 1. Frequency of the YST-motif (homo- and heterozygous) in females with juvenile idiopathic arthritis (JIA)

	YST +	YST -	Total
JIA-associated uveitis	135 (99%)	1 (1%)	136
JIA non-uveitis	179 (79%)	49 (22%)	228
Total	314	50	364

Ocular fluid analysis in childhood uveitis

In order to better understand the disease mechanism that distinguish chronic anterior uveitis in JIA from other forms of childhood uveitis, we measured a panel of 51 soluble mediators in

AqH (n=73) and paired serum (n=66) samples by multiplex immunoassay (**chapter 4**). In detail, we compared the soluble mediators of 21 children with JIA-associated uveitis, 15 with chronic anterior uveitis without arthritis (termed 'CAU'), 29 children suffering from noninfectious idiopathic uveitis, and 8 children with non-inflammatory conditions were used as unaffected controls. After correcting for disease activity and treatment, the levels of interleukin-29 (IL-29)/interferon-λ1 (IFNλ1) were specifically decreased in AqH from JIA-associated uveitis patients compared to idiopathic uveitis. No other mediators could distinguish JIA-associated uveitis from other forms of uveitis by univariate analysis. The area under the *receiver operating characteristic* (ROC) curve of 0.954 revealed that IL-29/IFNλ1 could perfectly differentiate JIA-associated uveitis from idiopathic uveitis. Likewise, using a cut-off of value of 22.7 pg/mL for IL-29/IFNλ1, JIA-associated uveitis could be relatively well distinguished (AUC 0.771) from chronic anterior uveitis without arthritis (CAU). Considering multiple mediator profiles, JIA-associated uveitis patients showed additional subtle disease specific changes in AqH (profiles) compared to the other groups investigated (**Figure 3** in chapter 4). IL-29/IFNλ1 could not be detected in the serum samples.
In conclusion, IL-29/IFNλ1 was found to be an intra-ocular biomarker for JIA-associated uveitis, suggesting that IL-29/IFNλ1 signaling might be a specific and important biomarker in JIA-associated uveitis.

Quality of Life in adults with JIA
In **chapter 5** we studied the impact of uveitis on the QoL of adolescent JIA patients, by analyzing their scores of three validated QoL questionnaires. Scores of 31 JIA-associated uveitis and 51 JIA without uveitis patients were compared. The vision-related QoL in JIA was lower in uveitis patients, despite 'good visual acuity' according to the criteria of the *world health organization (*WHO) and *standardization of uveitis nomenclature* (SUN) international working group.[1,2] According to these combined criteria, a patient with a bilateral best corrected visual acuity (BCVA) of 20/50 or worse is considered as visually impaired or legally blind (BCVA of 20/200 or worse and/or a visual field of 10˚ or less). Although, the general QoL scores did not differ between uveitis and non-uveitis JIA patients, the use of systemic immunomodulatory treatment (IMT) did negatively influence general QoL scores in JIA patients.
In conclusion, vision related QoL scores were lower in uveitis patients compared to non-uveitis JIA patients. The use of systemic IMT decreased general QoL scores in all JIA patients.

Prognosis of JIA-associated uveitis in adulthood
Chapter 6 describes a retrospective multicenter cohort study, outlining uveitis activity, complications and visual prognosis of 67 young adult patients with exclusively (childhood onset) uveitis and JIA. Since treatment strategies drastically changed in the early nineties with the advent of Methotrexate (MTX) and later anti-TNFα therapy (around the year 2000), and the ophthalmologic screening protocols for JIA were also introduced during that time, we compared patients with uveitis onset before and after 1990. Disease characteristics were studied at three fixed time-points; at the age of 18, 22 and 30 years. We found that the binocular visual outcome in adulthood is fairly good, but up to 30% of the JIA-associated uveitis patients developed severe visual impairment or blindness of at least one eye. Also, many patients needed ongoing treatment, had persisting uveitis activity and needed surgery during adulthood. Furthermore, more patients used systemic IMT when diagnosed with uveitis after 1990 compared to those diagnosed before 1990.

In conclusion, bilateral visual outcome of JIA-associated uveitis in adulthood is fairly good, but about one third of all adult JIA-associated uveitis patients developed one visually impaired or blind eye. Also, a fair amount of the patients had ongoing uveitis activity or needed treatment during adulthood as well as surgical interventions.

DISCUSSION AND FUTURE PERSPECTIVES

Uveitis associated with JIA is a serious and sight-threatening childhood disease, whose aggravating impact on prognosis and vision-related QoL stretches far into young adulthood. There is the unmet need to specifically predict the occurrence of uveitis in JIA in advance, and to unravel the complex disease mechanism in order to improve and standardize clinical care with the aim to use this as a framework for personalized treatment.

Prediction of uveitis in JIA

The introduction of periodic ophthalmologic screening in the nineties has considerably improved the early detection of uveitis in JIA patients. Yet, these existing tools still have several limitations. For example, current ophthalmologic screening programs provide a burden for JIA patients that never develop uveitis at all. In addition, despite careful monitoring of the anterior segment by regular slit lamp examinations, patients with uveitis usually experience delayed diagnosis. This illustrates the need for additional objective (clinical and molecular) markers that more accurately predict patients at risk and consequently aid in the design of personalized uveitis screening programs for JIA patients.

Results from the studies described in this thesis might alleviate some of these constrains. For example, in the genetic analysis we observed that nearly (if not all) female patients with uveitis carried at least one *HLA-DRB1* allele encoding the YST-motif (**Table 1**). Under the assumption that this association implies that the YST-motif is a prerequisite for the development of uveitis, the absence of this motif may render a female JIA patient at lower risk and consequently a less intense screening program might be scheduled. Theoretically, as a diagnostic test for uveitis in females, the negative predictive value of the absence of the YST-motif would reach 100%. If these promising results hold up in larger prospective cohort studies, we believe this genetic marker provides the first objective biomarker that can alleviate or potentially prevent ophthalmological screening in female JIA patients in advance (e.g. at JIA onset). Since we found this novel association with JIA-associated uveitis, it is tempting to speculate on the functional role of this motif in the underlying mechanisms of other forms of childhood uveitis, especially with the phenotypically similar CAU (without arthritis).

Furthermore, we discovered that an elevated ESR value, which is already routinely measured at JIA presentation, significantly increases the risk for uveitis. Therefore, in line with the above suggested design of personalized uveitis screening programs, patients with elevated ESR levels at JIA onset should be screened more frequently, particularly in the first year after JIA onset. Similar to the promising genetic results, clinical implementation of ESR requires future validation studies to more accurately determine its predictive value. However, the routine measurement of ESR greatly facilitates the design of large multi-center studies to adequately investigate and translate the use of this predictive marker into the clinics in the near future.

In addition to these encouraging genetic and blood markers, we also identified molecular markers associated to JIA-associated uveitis in eye fluid (AqH). However, the use of IL-29/IFNλ1 to predict the occurrence of uveitis in JIA is complicated by several factors. First of all, the levels of soluble mediators, such as cytokines, may reflect the current state of disease and theoretically, it would be interesting to determine if the levels of these mediators are also affected prior to clinical eye disease. This is partly based on the observation that some CAU patients, the typical chronic anterior uveitis without arthritis, displayed 'normal' IL-29 levels and later developed arthritis (i.e. 'became' JIA patients). Another complicating factor is

that the difference in the levels of expression for IL-29 between patients and controls are near the usual inter-assay variability of multiplex technology, which would make classification based upon the proposed optimal discriminative cut-off value highly challenging on an individual basis (for diagnosis). Most importantly, since it is unethical to draw AqH from JIA patients without any ocular manifestations, we were unable to compare the levels of this mediator to eyes of JIA patients without uveitis.

To overcome the latter limitation, less invasive techniques are required, to study and compare conditions within the anterior chamber of the eye at JIA onset. These techniques could serve as possible diagnostic instruments for uveitis in JIA patients; *Raman spectroscopy* is described as such a noninvasive technique with the ability to quantify biological molecules within the eye.[3,4] Unfortunately, this technique is still in its infancy and is far off before being used as a diagnostic tool. Regardless, it would provide an interesting tool for the discovery of additional molecular markers of uveitis in JIA and can be used to also map molecular content in non-affected (JIA) eyes in future studies. Another emerging technology for studying JIA eyes is the *laser flare-cell photometry*, which is a relatively new and noninvasive technique to quantitatively measure flare (in other words the 'reflection of light from proteins in the AqH') and cells within the AqH.[5,6] Although the laser flare photometer is already clinically used to objectively measure disease activity in uveitis patients, it has not been applied as a diagnostic tool for screening for uveitis at JIA onset. Interestingly, a Japanese study of psoriasis patients found higher flare in aqueous of patients – including patients without any eye symptoms – compared to healthy subjects, suggesting that the inflammation underlying psoriasis might cause subclinical disruption of the blood aqueous barrier.[5] Based upon these findings, we postulate that measuring AqH flare at JIA onset might aid in the prediction of the occurrence of uveitis. Another noninvasive way to study the ocular micro-environment, might be a study on the composition of tear fluid. In a recent study, Carreno and associates found different levels of cytokines and chemokines in tear fluid of patients with uveitis compared to healthy controls.[7]

Possible disease mechanisms of JIA-associated uveitis

Understanding the disease mechanism of JIA-associated uveitis is crucial for improvement of the current, often insufficient and impactful, treatment strategies. However, the complex multifactorial character makes understanding of the disease highly challenging. Based upon the results of the studies in this thesis, it is tempting to connect our observations in the light of the several interesting and related studies. For example, high anti-parvovirus B19 antibody titers were found in AqH of JIA-associated uveitis patients.[8] Here, we linked the YST-motif in the *HLA-DRβ1* protein and lower AqH levels for IL-29/IFNλ1 to JIA-associated uveitis. Interestingly, the YST-motif and IL-29/IFNλ1 are both involved in parvovirus B19 infection control, which makes it tempting to speculate that immunity to parvovirus B19 plays may play an important role in the pathogenesis of JIA-associated uveitis.[9,10] Several hypothetical mechanisms could underlie this interesting association. First of all, parvovirus B19 may directly induce JIA-associated uveitis by concealed eye infection. However there are several arguments against this hypothesis; First of all, the hallmark insidious disease course of JIA-associated uveitis is highly atypical for infectious uveitis. Secondly, to the best of our knowledge, parvovirus DNA cannot be detected during clinical disease, and, finally, not all patients with JIA-associated uveitis display antibodies to this virus in AqH. The association with the YST-motif may therefore suggest an alternative mode of action: An immune response to control an infection by parvovirus B19 via peptide presentation by *HLA-DRβ1*

with YST+ individuals is (highly) successful but escalates into derailed (continuous) immune responses. In support of this, we observed that the YST-motif, which resides in the peptide binding groove of *HLA-DR*, influences peptide preferences of *HLA-DR*. However, the *in silico* predictions should be functionally ascertained by *in vitro* binding assays and ideally by functional *in vivo* (HLA transgenic) models. This second mechanism (the 'derailed immune response'), might be preceded by disruption of the blood-ocular barrier in JIA-associated uveitis, which normally also partly functions to extinguish immune responses in immunogenic eye tissues after an infection has been controlled. This latter hypothesis is supported by emerging evidence of the importance of IL-29/IFNλ1 in barrier functions, where decreased levels of IL-29/IFNλ1 deteriorate barrier function. Thus, the lower AqH of IL-29/IFNλ1 may reflect disruption of the blood-ocular barrier in JIA-associated uveitis. IL-29/IFNλ1 is the main cytokine of the type III interferons, which signal through their receptor (IFNLR1) expressed mainly by epithelial and immune cells.[11,12] IL-29/IFNλ1 is crucial for tightening of the blood-brain barrier permeability by regulating microvascular endothelial cells to inhibit viral infection and for regulation of the immune cell-epithelial interface.[12] Intriguingly, Galani and colleagues recently studied the role of IFNs in influenza virus infection in mice.[13] They found IFNλs (including IL-29/IFNλ1) to be the first line defense in viral infection that operate mostly without activating general inflammatory pathways (lack of typical 'inflammation'). IFNλ was also found to be a central regulator of mucosal immunity, suppressing intestinal inflammation.[14] Interestingly, intestinal inflammation due to gut dysbiosis has very recently been implicated in a wide variety of auto-inflammatory and autoimmune diseases.[15] Therefore, it might be interesting to study the microbiome of JIA patients, in order to understand its role in the development of uveitis (as well as JIA) or determine if microbiome studies may aid in identification of accessible molecular markers for the prediction of uveitis.

The association of ANA's, the correlation of ANA-positivity with the presence of the YST-motif, the presence of plasma cells in iridectomy samples, and beneficial effects of Rituximab in reducing eye inflammation in patients suggest B-cell involvement in the pathogenesis of JIA-associated uveitis.[16,17] Interestingly, also parvovirus B19 infection targets B-cells.[18] Furthermore, MTX is known to suppress uveitis activity in JIA patients. The exact working mechanism of MTX in alleviating JIA-associated uveitis remains unsolved, but we do know that MTX is essential in the reduction of anti-biological antibody formation in Adalimumab treated patients, thus, suppression of antibody (e.g. ANA) producing B cells may be among the beneficial effects of MTX in treatment of JIA-associated uveitis, further supporting an important role for B-cells in JIA-associated uveitis.[19] Antibodies to parvovirus B19 and ANA are linked to JIA-associated uveitis, yet the production of these two humoral mediators may be related to a recently elucidated process underlying affinity maturation in germinal centers. A recent study by Degn and associates demonstrated that a single (auto)reactive B cell clone may promote conditions that lead to expansion of a multitude of unrelated autoreactive B cells under diseased conditions (loss of tolerance), which ultimately leads to the production of various serum autoantibodies.[20] This raises the possibility that the generation of ANAs are not the result of previously postulated 'molecular mimicry' with structurally related pathogenic/environmental antigens (such as parvovirus B19), but 'collateral damage' due to inflammatory conditions that promote the expansion of autoantibody-producing B cells. One strategy to identify the breadth of auto-antigens in JIA-associated uveitis may be via antibody microarrays to specify to which antigens ocular and serum autoantibodies are directed.

Treatment timing in JIA-associated uveitis

High ESR indicates a (nonspecific) elevated state of inflammation in the patient's body. This is interesting, since no association between arthritis activity at JIA onset and the susceptibility to uveitis has yet been described. This elevated 'state of inflammation' at JIA onset may lead to disruption of the blood aqueous barrier and JIA-associated uveitis.[21] Recent insight into early aggressive treatment in JIA-associated uveitis revealed that this reduces disease activity and the formation of sight-threatening complications, an important clinical observation that may be extrapolated to aggressive treatment at JIA onset to prevent the development of uveitis. Also, the fact that laser flare values might stay elevated after uveitis onset, despite of the absence of anterior chamber cells advocates for aggressive treatment at JIA-onset. In line with this, Kostik and associates observed a profound decrease in the incidence of uveitis in JIA in patients treated with MTX from baseline/onset (11.5% vs. 46.7% in those not treated with MTX), but this study had a follow-up of only 2 years.[22] The question remains whether early treatment prevents or merely postpones the development of uveitis. Ideally, monitoring patients from baseline with an extended follow-up and report development of uveitis, and compare these to previous observations of Heiligenhaus and associates, would informs us about the impact of current treatment on uveitis development.[23] All JIA patients, from secondary and tertiary medical centres, should be included in such an incidence study. This asks for good (at first) national collaboration between rheumatologists and ophthalmologists, as discussed later in this chapter.

Unfortunately, bearing in mind our finding that the use of systemic IMT was increased in the last decades and the finding that use of systemic IMT reduces risk of vision loss, QoL scores were negatively affected by the use of systemic IMT.[24] Furthermore, not all patients (directly) respond to the first or second line treatments, and consequently, prolonged periods and/or various hazardous drug schemes must be attempted before uveitis activity is manageable - during which irreversible vision loss may have already occurred. This illustrates the need to expand the therapeutic armamentarium for JIA-associated uveitis, which ideally would be tailor-made for individual patients following the philosophy of *'personalized medicine*' and for biomarkers that can predict treatment response.

A systems medicine approach to JIA-associated uveitis

It is important to note that the in this thesis identified genetic, clinical and molecular markers for JIA-associated uveitis is by no means exhaustive. In fact, we would like to emphasize that a wide variety of complex interaction of multiple biological layers of the immune system are most likely involved in the development of uveitis – in addition to the already intricate molecular background of JIA. To fully cover the wide spectrum of a multitude of contributing epigenomic factors paving the way for uveitis in JIA, a more 'holistic' experimental approach would be required; '*Systems Medicine*' is such an approach, which exploits a multitude of strategically exploited 'OMICS' layers (e.g. *transcriptome, epigenome, proteome, and metabolome* of individual immune cell subsets or serum/plasma) and ultimately integrates these data layers with high performance computational modelling to an underlying network of nodes leading to disease.[25] Systems medicine is emerging as a translational extension of *systems biology* and through this, we can possibly find attractive targets to treat JIA-associated uveitis and exploit the biological fingerprints to identify more robust biomarkers to predict uveitis or treatment response, in advance. Here, the design of more accurate uveitis-prediction models would most likely benefit from 1) an increase in the number of

associated biomarkers and 2) appropriate methods that take into account the dynamics of multi marker-profiles (e.g. a panel of the previously discovered clinical and biological markers such as transthyretin, S100A8/A9, anti-histone antibodies, soluble IL-2 receptor and amyloid A1 over time) in a prediction model to design personalized ophthalmologic screening.[26] To better understand the disease mechanism of uveitis, we propose to profile multiple biological informative 'layers' of the immune system in JIA patients with and without uveitis, followed by computational integration and mining the data to unravel the molecular origins of disease. Such studies would benefit from including an interesting additional patient group: As we found in our AqH analysis, the clinically nearly identical JIA-associated uveitis and CAU showed partly overlapping AqH profiles (**Figure 3**, chapter 4). This indicates molecular similarities between these two entities (and the fact that these conditions are clinically near identical except for the lack of arthritis in CAU patients). Therefore, including CAU patients in these systems medicine analyses would aid in the understanding of the disease mechanism of both JIA-associated uveitis and CAU. There are, however, unique challenges with studying these childhood conditions: Although the advent of single cell profiling technologies has dramatically reduced the required amount of patient material, at present, the in-depth study of multiple molecular layers demands the collection of relatively large amounts of patient material (e.g. blood, AqH). This is challenging due to obvious ethical issues (burden and relatively large amount of material needed) in scientific studies with children. In light of this potential limitation, systems medicine in childhood diseases imposes additional challenges that can be overcome by strategically aligning the non-hypothesis driven 'systems approach' to the state-of-the-art in the pathogenesis of JIA-associated uveitis. Based upon the above described emerging role of B-cells in the pathogenesis, studying e.g. the transcriptome and epigenome of this cell subset may provide an exciting stepping stone deeper down the rabbit hole of the molecular universe of JIA-associated uveitis.

Sex differences in JIA-associated uveitis

Previous clinical studies have revealed evidence for differences in clinical presentation and disease course between boys (usually more severe) and girls with JIA-associated uveitis.[27] By performing sex-stratified association analysis we observed that the overall association at *HLA-DRB1* was exclusively driven by females, and provides the first molecular evidence for sexual dimorphism in JIA-associated uveitis. This makes it tempting to speculate that sex hormone levels influence disease mechanisms in JIA-associated uveitis. Interestingly, the female hormones estrogen and prolactin promote survival of autoreactive B-cells and induce B-cell activation, which is in line with our discussion on B-cell involvement in the pathogenesis of JIA-associated uveitis.[28,29] Also, ANA positivity in JIA-associated uveitis is more frequently observed in girls compared with boys, indicating that this function of B-cells may particularly be important in female patients.[30] Despite the recent gender discussion in the society, this growing evidence of sexual dimorphism underlines that sex-stratification might be extremely important in future studies on JIA-associated uveitis, which might also be true for JIA studies in general.[31] These strong hints for sexual dimorphism make it reasonable to suggest that also the clinical management of uveitis in boys and girls may soon follow sex-specific recommendations in future guidelines. Curiously, this female specific genetic signature of JIA-associated uveitis may introduce a novel branch of sex-specific (or 'X-Y') medicine that bridges or parallels the era of the personalized medicine. To study sex-specific disease mechanisms and therapeutic responses in more detail, sufficient numbers of well-

characterized patients are necessary, underlining the importance of national and international collaboration to enable the collection of sufficiently powered patient cohorts.

Multidisciplinary and (inter)national collaboration in the management of JIA-associated uveitis

Since patients with JIA-associated uveitis suffer from a complex condition that affects multiple organs, a multidisciplinary approach for these patients is definitely required. Such collaboration between predominantly ophthalmologists and rheumatologists will offer patients the optimal standard of care. We found an important negative effect of treatment on the general QoL in all JIA patients, highlighting the importance of optimizing daily care. To achieve this, there is the need for national and international interdisciplinary guidelines to manage JIA-associated uveitis. Recently, the SHARE-project (*Single Hub and Access point for Pediatric Rheumatology in Europe*) was launched. Here, rheumatologists and ophthalmologists from various European institutes have joined forces to collectively develop recommendations for the management of JIA-associated uveitis, based on a standard evidence-informed consensus process. The first concept of SHARE guidelines are in press, and will hopefully be soon adapted by the rheumatology and ophthalmology fields.

Another consideration confirmed by results from our studies is the disease continuation into adulthood. This highlights in addition to multidisciplinary paediatric care, the need for a seamless transition from paediatric to adult care.

Despite several GWAS investigations into the genetic background of JIA, genome-wide studies specifically aimed at deciphering the genetic heritability of uveitis in this patient population were previously lacking.[32] This may seem surprising given the common occurrence of uveitis but such a comparison would demand years of extensive ophthalmological follow-up to confidently state if a patient developed uveitis, and proper data-sharing between pediatric rheumatologists and ophthalmologists.[23,33,34] Such extended and detailed clinical data is usually not directly available in large genetic studies and given the insidious onset, the use of self-report surveys would be highly unreliable. In addition to these requirements, the relatively rarity of this conditions makes extensive multicenter collaborations vital to the design of sufficiently powered studies. Feasibility of such tight collaborations within Europe is demonstrated in two genetic cohorts of 576 JIA-patients (214, 37% with uveitis). Our ambition is to expand this GWAS to a global investigation with the aim to identify additional common, modestly-penetrant variation underpinning JIA-associated uveitis (e.g. loci linked to B-cells or to the barrier function). Well documented clinical data on treatment responses or disease course may aid in the identification of loci associated with these clinical outcomes.[35]

Patient participation in research into JIA-associated uveitis

Besides the importance of collaboration between health professionals, the ultimate purpose is to serve and guide patients in coping and treating their conditions. In order to determine impact and priority of research in JIA-associated uveitis it is vital to include the 'patient's voice'.[36] An important instrument to monitor disease impact by direct input of patients is the use of QoL measurements. Although it may seem obvious that vision-related QoL is worse in patients with uveitis, such measurements help to objectify these assumptions and consequently can also be used to monitor treatment response (next to standardized ophthalmologic examinations) from the patients perspective view.[37] In order to develop a set of core outcome measures for clinical studies in JIA-associated uveitis, the *Multidisciplinary*

Working Group for Uveitis in Childhood (MIWGUC) was initiated.[38] Besides consensus about documentation on, among others, uveitis activity and complications, the collaborators came to an agreement that the use of QoL measurement is essential in clinical studies. Nevertheless, the vision-related QoL questionnaire for children (*Youngsters' Eyesight on Quality of Life (Eye-Q*)) is, at this moment, only available in the English language, but fortunately, non-English versions of the Eye-Q will soon be made available which also facilitates international collaborations.[39]

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Chapter 8

Nederlandse samenvatting

NEDERLANDSE SAMENVATTING

Introductie

Dit proefschrift gaat over *uveitis* (oogontsteking) die ongeveer in 30% van de gevallen van kinderen met *juveniele idiopathische artritis (JIA, jeugdreuma*) voorkomt. JIA is een gewrichtsontsteking die op kinderleeftijd ontstaat en waaraan 16-150 per 100.000 kinderen lijden. Het typische van uveitis bij JIA is dat de ontsteking zich voordoet in het anterieure (voorste) deel van het oog en het asymptomatisch begint. Echter wanneer er niet tijdig wordt behandeld, kunnen deze kinderen te maken krijgen met permanent verlies van hun visus (gezichtsvermogen). Om deze reden worden kinderen met JIA door de oogarts gescreend op uveitis.

Kinderen met JIA-geassocieerde uveitis worden regelmatig behandeld met immuun-modulerende medicijnen die nare bijwerkingen hebben (bijvoorbeeld Prednison). We weten dat JIA en uveitis auto-immuunaandoeningen zijn, waarbij verschillende factoren van het immuunsysteem, maar ook invloeden van buitenaf, een rol lijken te spelen. Het exacte ziektemechanisme is helaas nog niet volledig bekend en kennis hiervan is noodzakelijk om nieuwe behandelingsstrategieën en wellicht zelfs nieuwe medicijnen te kunnen ontwikkelen.

In **hoofdstuk 1** van dit proefschrift leggen we uit wat er op dit moment bekend is over de kenmerken, het beloop, de behandeling en het ziektemechanisme van JIA-geassocieerde uveitis. Wanneer kinderen met JIA uveitis ontwikkelen, gebeurt dit in de meeste gevallen (>90%) binnen 4 jaar nadat de diagnose JIA door de kinderreumatoloog gesteld is. De afgelopen jaren zijn er verschillende aanknopingspunten gevonden die uveitis bij kinderen met JIA kunnen voorspellen; zo weten we bijvoorbeeld dat kinderen die voor en tijdens hun 7^{e} levensjaar JIA hebben ontwikkeld, meer kans hebben op uveitis dan kinderen die na hun 7^{e} levensjaar JIA kregen. Op basis van deze voorspellers worden kinderen met JIA 1 tot 4 keer per jaar tot hun 18^{e} verjaardag oogheelkundig gecontroleerd. Ondanks de verschillende voorspellers en controles, blijft het een uitdaging om uveitis bij kinderen met JIA tijdig te ontdekken. Daarentegen worden veel kinderen met JIA die nooit uveitis zullen ontwikkelen onnodig door de oogarts onderzocht. Dit is onwenselijk, omdat het oogheelkundig onderzoek door veel kinderen als belastend wordt ervaren.

We weten ook dat JIA en uveitis auto-immuun ziektes zijn, en specifieke afweercellen (T-helper 1 en T-helper 17 cellen) een rol spelen in het ziektemechanisme. De exacte werking hiervan is nog onbekend. In het voorste oogkamervocht kunnen we afweercellen en eiwitten bestuderen, zodat we het ziektemechanisme van uveitis beter kunnen begrijpen. Er zijn recent *grote genetische studies* bij JIA-patiënten gedaan waarbij specifieke loci (plekken in het DNA) zijn ontdekt, die een rol lijken te spelen bij het ontstaan van JIA. Zulke genetische studies zijn nog niet gedaan voor JIA-geassocieerde uveitis. Het zou interessant zijn om het DNA van JIA-patiënten met en zonder uveitis met elkaar te vergelijken met een genetische studie waarbij het hele genoom wordt bestudeerd (een GWAS). Tevens zijn er studies gedaan naar de kwaliteit van leven en de prognose van uveitis gedurende de kinderleeftijd. Echter, we weten niet hoe het de patiënten op volwassen leeftijd vergaat.

Er is dus vraag naar klinische, genetische en moleculaire factoren (*biomarkers*) die het ontstaan van uveitis bij JIA-patiënten kunnen voorspellen en het ziektemechanisme beter doen begrijpen. Daarnaast zijn er studies nodig die de kwaliteit van leven en prognose van patiënten met JIA-uveitis op volwassen leeftijd uiteenzetten. Om bovenstaande redenen bestudeerden we in dit proefschrift het voorste oogkamervocht, het DNA,

ontstekingsparameters in het bloed en klinische factoren van patiënten met JIA-geassocieerde uveitis en hebben we de prognose en kwaliteit van leven van jongvolwassenen met JIA-geassocieerde uveitis onderzocht.

Klinische voorspellers voor het ontstaan van uveitis in JIA

In **hoofdstuk 2** was ons doel om ontstekingsparameters in het bloed te vinden die het ontwikkelen van uveitis bij kinderen met JIA kunnen voorspellen. In dit retrospectieve onderzoek hebben we de klinische gegevens van 147 JIA-patiënten met en 211 JIA-patiënten zonder uveitis bestudeerd. Eén van de bevindingen was dat de bezinking (*BSE, bezinkingssnelheid erytrocyten*) op het moment van het ontstaan van JIA hoger was bij patiënten met uveitis, dan bij patiënten zonder uveitis. De bezinkingswaarde is vaak verhoogd bij diverse auto-immuunziekten. Een tweede bevinding was dat het ontstaan van JIA op een jongere leeftijd, en de aanwezigheid van *anti-nucleaire antistoffen* (ANAs) de kans op het ontstaan van uveitis bij JIA-patiënten vergrootten. Deze laatste voorspellers werden ook al in eerdere wetenschappelijke onderzoeken aangetoond. Om de nieuwgevonden factor, bezinking, in de praktijk te kunnen toepassen, hebben we alle gevonden voorspellers samengenomen en het risico op het ontstaan van uveitis in verschillende situaties in een grafiek weergegeven (zie **Figuur 1** in **hoofdstuk 2**).

We kunnen concluderen dat een verhoogde bezinkingswaarde op het moment van het ontstaan van JIA, het ontwikkelen van uveitis in de toekomst kan voorspellen. Omdat de bezinking standaard bij kinderen met JIA gemeten wordt zodra ze bij de kinderreumatoloog komen, kunnen we deze voorspeller eenvoudig gebruiken in de dagelijkse praktijk.

Erfelijke factoren in JIA-geassocieerde uveitis

In **hoofdstuk 3** hebben we een *genoombrede associatiestudie* (GWAS) gedaan, waarbij we genetische variaties in het hele genoom (DNA) hebben bestudeerd en vergeleken tussen 192 JIA-patiënten met uveitis en 330 JIA-patiënten zonder uveitis. Het DNA was afkomstig van patiënten uit Nederland, Duitsland, België en Zwitserland. We hebben ontdekt dat een specifiek *aminozuur-motief* (bouwstenen voor eiwitten) significant vaker voorkomt bij patiënten met uveitis. Het gaat hier om het YST-motief in het gen dat codeert voor het HLA-DR eiwit. HLA-genen spelen een belangrijke rol in de communicatie van het afweersysteem. Het YST-motief bevindt zich op het eiwit-bindende deel van HLA-DR. Met een computermodel hebben we getest of het YST-motief van invloed is op het binden van eiwitten (*peptiden*). Uit deze test is gebleken dat HLA-DR met het YST-motief eiwitten anders bindt dan HLA-DR zonder dit uveitis motief – met mogelijke gevolgen voor het ziekte mechanisme.

Interessant is dat de associatie met het YST-motief alleen bij vrouwen werd gevonden. Alle vrouwen met uveitis, met uitzondering van één vrouw, hadden het YST-motief (zie **tabel 1** in **hoofdstuk 7**). De enige vrouw die het YST-motief niet had, bleek een andere vorm van uveitis te hebben dan de karakteristieke asymptomatische uveitis anterior, zoals we dit bij JIA zien.

Hiermee concluderen we dat het YST-motief in HLA-DR, vaker voorkomt bij vrouwen met JIA en uveitis dan bij vrouwen met JIA zonder uveitis. De binding van eiwitten aan het HLA-DR eiwit bleek afhankelijk te zijn van het YST-motief. Eiwit presentatie door HLA-DR zou een belangrijke rol kunnen spelen bij het ziektemechanisme van uveitis bij JIA.

De analyse van oogvocht bij kinderen met uveitis

Om het ziektemechanisme van JIA-geassocieerde uveitis beter te kunnen begrijpen, hebben we in **hoofdstuk 4** voorste oogkamervocht bestudeerd. We vergeleken 51 mediatoren in het voorste oogkamervocht van kinderen met verschillende vormen van uveitis. Van 73 kinderen konden we tijdens een voor klinische doeleinden geplande operatie of diagnostische voorste oogkamer punctie oogvocht afnemen. Van deze 73 kinderen was bij 21 kinderen JIA-geassocieerde uveitis vastgesteld en hadden 15 kinderen eenzelfde chronische anterieure uveitis maar zonder JIA. Daarnaast hadden 29 kinderen intermediaire of posterieure uveitis (midden- of achterin het oog) en hadden 8 kinderen geen uveitis.

Eén van de mediatoren, *interleukine-29 (IL-29)/interferon-λ1 (IFNλ1),* bleek in significant lagere concentraties aanwezig te zijn bij JIA-geassocieerde uveitis in vergelijking met andere vormen van uveitis en kinderen zonder uveitis. Met behulp van *IL-29/IFNλ1* kon JIA-geassocieerde uveitis erg goed onderscheiden worden van intermediaire en posterieure uveitis en redelijk goed van chronische anterieure uveitis zonder JIA. We hebben een profiel gemaakt waarin we behalve *IL-29/IFNλ1,* ook andere mediatoren in het voorste oogkamervocht hebben meegenomen (**Figuur 3** in **hoofdstuk 4**). Uit dit profiel is gebleken dat JIA-geassocieerde uveitis andere kenmerken heeft dan uveitis intermediaire en posterieure uveitis, maar vrij veel overeenkomsten heeft met chronische anterieure uveitis zonder JIA. We hebben ook serum (bloed) van de patiënten geanalyseerd, maar hierin hebben we *IL-29/IFNλ1* niet kunnen detecteren.

We kunnen concluderen dat *IL-29/IFNλ1* in een lagere concentratie voorkomt in de ogen van patiënten met JIA-geassocieerde uveitis, wat erop kan duiden dat deze factor een rol speelt in het ziektemechanisme.

Kwaliteit van leven bij volwassenen met JIA

Bij patiënten met JIA die inmiddels volwassen geworden waren, hebben we drie verschillende vragenlijsten over de kwaliteit van leven afgenomen. In **hoofdstuk 5** vergeleken we de uitkomsten hiervan tussen JIA-patiënten met (31 patiënten) en zonder uveitis (51 patiënten). De uveitis-patiënten, waarvan het merendeel volgens de huidige criteria* een 'goede visus' had, bleken toch lager te scoren op de visus-gerelateerde kwaliteit van leven vragenlijst. De algemene kwaliteit van leven verschilde niet tussen patiënten met en zonder uveitis. Wel bleek dat het gebruik van immuun-modulerende medicijnen, de algemene kwaliteit van leven bij alle JIA-patiënten negatief beïnvloedde.

We kunnen concluderen dat de visus-gerelateerde kwaliteit van leven lager was bij volwassen JIA-patiënten met uveitis dan bij JIA-patiënten zonder uveitis. Tussen deze groepen was de algemene kwaliteit van leven gelijk. Wel werd de algemene kwaliteit van leven negatief beïnvloed door het gebruik van immuun-modulerende medicatie bij alle JIA-patiënten.

De prognose van JIA-geassocieerde uveitis op volwassen leeftijd

In **hoofdstuk 6** beschreven we hoe het gaat met patiënten met JIA-geassocieerde uveitis zodra zij volwassen zijn geworden. We bestudeerden de ziekteactiviteit, complicaties en de visus van 67 jongvolwassenen. We hebben in deze analyses patiënten die voor en na 1990 uveitis ontwikkelden met elkaar vergeleken. Deze vergelijking hebben we gemaakt, omdat er in de jaren 90 het nieuwe effectieve medicijn *Methotrexaat* (MTX) voor JIA-geassocieerde uveitis op de markt kwam. Daarbij werden enkele jaren later ook de '*Biologicals*' (zoals *Adalimumab*) voor de behandeling van JIA-geassocieerde uveitis toegepast.

**De criteria voor visusverlies van de world health organization (WHO) en van de internationale uveitis werkgroep.*

We vonden dat de binoculaire visus (kijken met twee ogen) eigenlijk erg goed was, maar dat ongeveer 30% van de patiënten op volwassen leeftijd een ernstig verlaagde visus had aan één oog. De visus leek iets beter te zijn voor patiënten die uveitis na 1990 hadden ontwikkeld, in vergelijking met patiënten die uveitis voor 1990 hadden ontwikkeld, echter dit verschil konden wij niet statistisch aantonen. Veel patiënten werden op volwassen leeftijd nog behandeld (medicatie en operaties) en in de helft van de gevallen was de ontsteking nog actief. Patiënten gebruikten significant vaker immuun-modulerende medicatie als hun uveitis na 1990 ontstaan was.
Dus de binoculaire visus van jongvolwassenen met JIA-geassocieerde uveitis is vrij goed, maar ongeveer 1/3 van de patiënten had één slechtziend of blind oog ontwikkeld. Ook had een groot deel van de patiënten nog te maken met ziekteactiviteit of waren er medicatie of operaties nodig om hun 'kinderziekte' op volwassen leeftijd te behandelen.

Chapter 9

Dankwoord
Curriculum Vitae
List of publications

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CURRICULUM VITAE

Anne-Mieke Haasnoot was born on the 8th of November 1988 in Rotterdam, the Netherlands, and grew up in Oostvoorne. After finishing her bilingual gymnasium at the Penta College CSG Jacob van Liesveldt in Hellevoetsluis, she started medical school in 2007 at the University of Utrecht. During medical school she also participated in the student rowing team and won the national rowing contest in the novice female eight and the silver medal at the European Student Championship for rowing in 2010.
In November 2013 she graduated and subsequently started her PhD-project on uveitis associated with juvenile idiopathic arthritis (JIA) under the supervision of Prof. dr. J.H. de Boer, Prof. dr. T.R.D.J. Radstake and Dr. J.J.W. Kuiper at the department of Ophthalmology and Laboratory of Translational Immunology of the University Medical Center (UMC) Utrecht. During her PhD she participated in the SHARE-project to develop international recommendations for the management of JIA-associated uveitis. In 2015 she won the 'poster prize' for the work on ocular fluid (Chapter 4) at the UMC Utrecht science day of Surgical Specialties. In 2017 her oral presentation on the outcomes of the genome wide association study (Chapter 3) was awarded 'most innovative' at the congress of the International Ocular Inflammation Society in Lausanne. In 2016 she was co-applicant in a grant proposal for future research on JIA-associated uveitis, which was successfully assigned.
In 2016 she participated in the Ride New York biking team of the ophthalmology department in the UMC Utrecht and raised money for Stichting Niet Blind, and she joined the pediatric rheumatologists of the UMC Utrecht on the first day (cycling to Aachen) of their cycling tour to Genua to draw attention to research in JIA. Since 2015 she is a trainer at the cycling sport club in Utrecht, participated in the trainers committee, and cycled several cyclo's (e.g. Trois Ballons, Marmotte).
In 2018 she will start as an ophthalmology resident at the UMC Utrecht.
Anne-Mieke is married with Aike Munneke and together they have a daughter, Jasmijn (2017).

LIST OF PUBLICATIONS

1. **Haasnoot AJ,** van Tent-Hoeve M, Wulffraat NM, Schalij-Delfos NE, Los LI, Armbrust W, Zuithoff NP, de Boer JH. Erythrocyte sedimentation rate as baseline predictor for the development of uveitis in children with juvenile idiopathic arthritis. Am J Ophthalmol. 2015 Feb;159(2):372-7.e1.

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3. **Haasnoot AJ**, Vernie LA, Rothova A, V D Doe P, Los LI, Schalij-Delfos NE, de Boer JH. Impact of juvenile idiopathic arthritis associated uveitis in early adulthood. PLoS One. 2016 Oct 10;11(10):e0164312.

4. **Haasnoot AJ**, Sint Jago NF, Tekstra J, de Boer JH. Impact of uveitis on quality of life in adult patients with juvenile idiopathic arthritis. Arthritis Care Res (Hoboken). 2017 Dec;69(12):1895-1902.