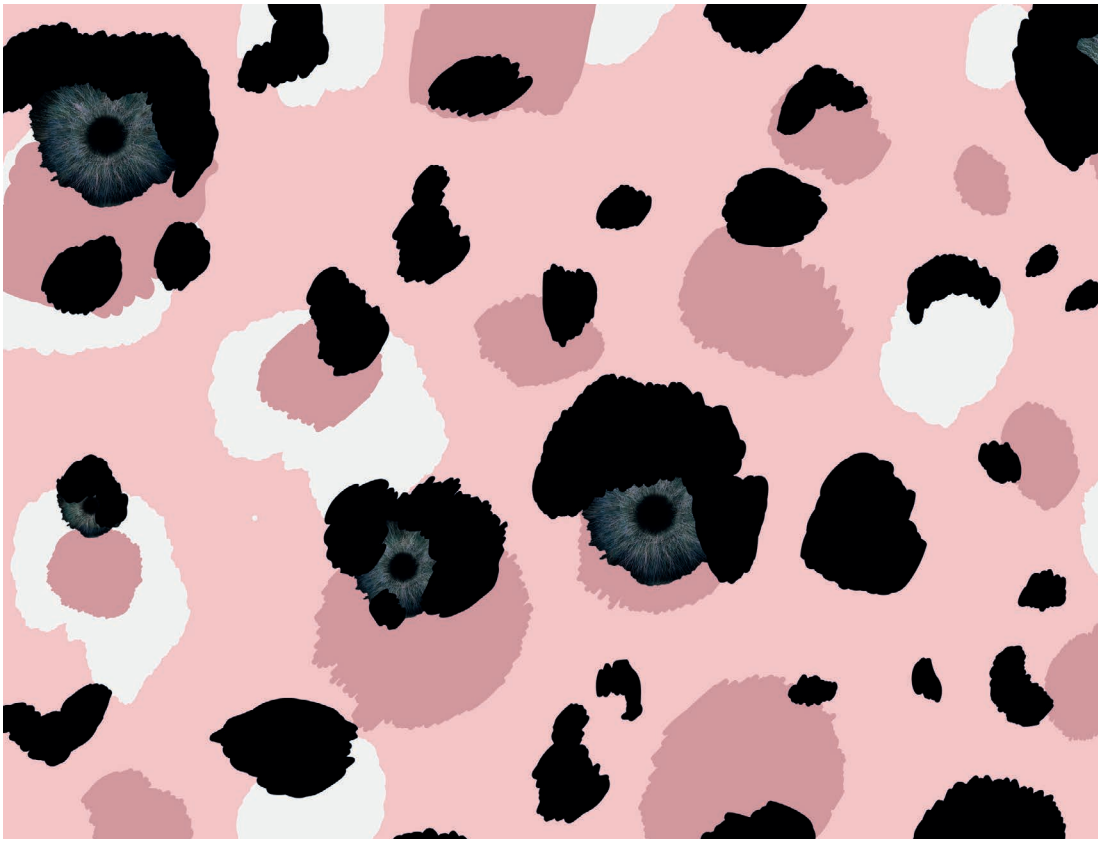
# Deciphering the impact of childhood non-infectious uveitis:

## a clinical, genetic and immunological framework for personalized medicine



Roos Wennink

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**Colofon**



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# Deciphering the impact of childhood non-infectious uveitis:

*a clinical, genetic and immunological framework for personalized medicine*

**Het ontrafelen van de impact van uveïtis bij kinderen:**
*Een klinisch, genetisch en immunologisch raamwerk voor patiëntgerichte zorg*
(met een samenvatting in het Nederlands)

**Proefschrift**

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**Roos-Anne Wibine Wennink**

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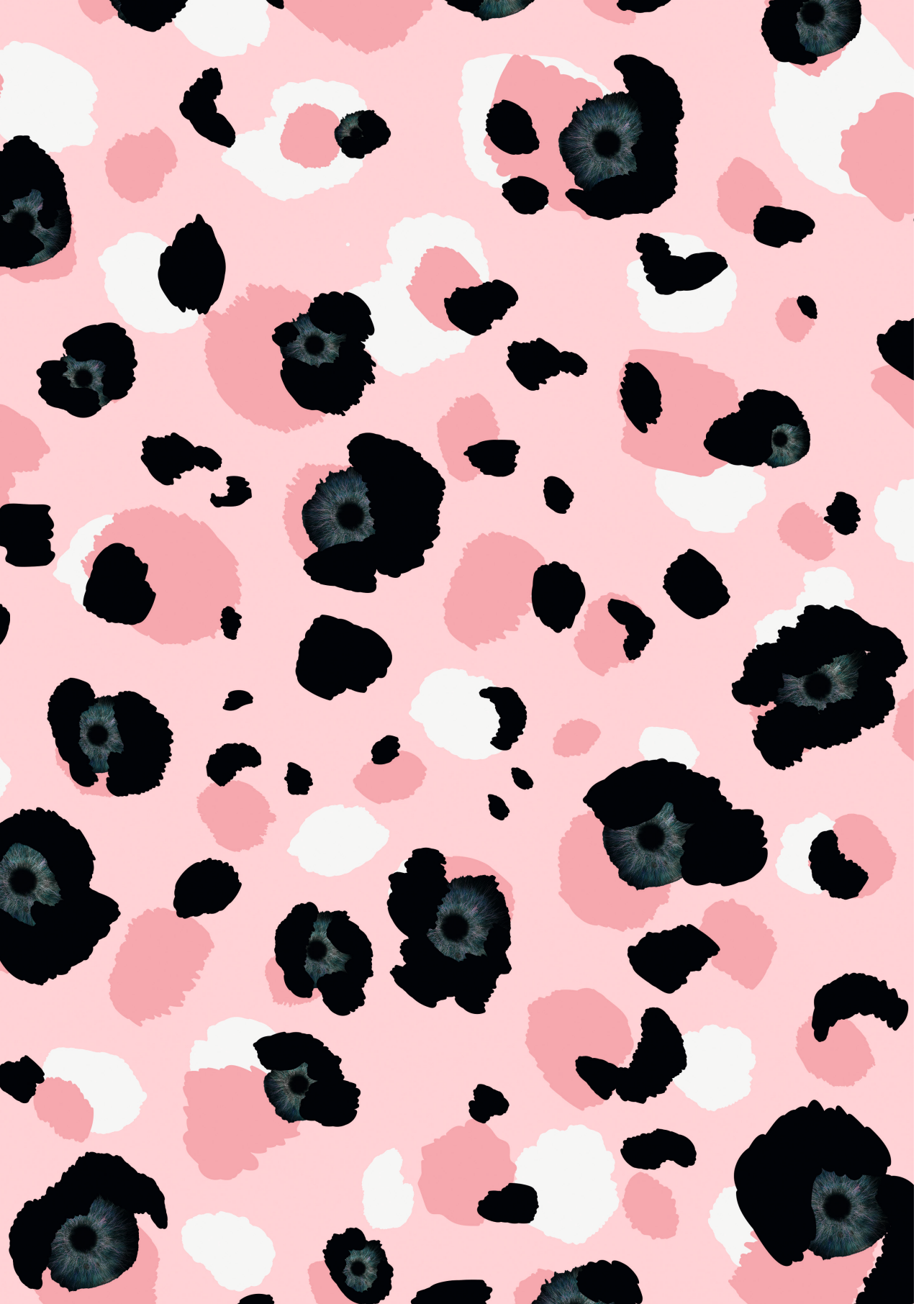
# Contents



# CHAPTER 1

## General introduction and aims of this thesis

## Background and epidemiology of childhood uveitis

Uveitis is a severe and potentially blinding eye disease and is characterized by inflammation of the iris, choroid and retina. The occurrence of uveitis in children (onset before the age of 16) is rare and accounts for 5-10% of all uveitis patients.[1] The estimated incidence of pediatric uveitis is 4.3 per 100,000 with variations among countries and ethnic populations.[2] According to the *Standardization of Uveitis Nomenculture* (SUN) guideline, the anatomic site of the inflammation determines the classification of uveitis: anterior uveitis, intermediate uveitis, posterior, or if multiple anatomical locations are involved - panuveitis (**Figure 1**).[3]

In a minority of pediatric uveitis patients (6-33%) an infectious cause can be identified. [4] In all other patients, the type of uveitis is classified as "non-infectious". Various lines of evidence strongly support that this type of uveitis is immune-mediated: the vast majority of patients develops uveitis in conjunction with a systemic inflammatory disease, such as uveitis associated with juvenile idiopathic arthritis (JIA-uveitis) that accounts for about half of cases (41-67%).[5] In the other half of cases uveitis typically manifests as an isolated idiopathic inflammatory condition.[6,7] **Figure 1** illustrates the anatomy of the eye and the anatomical classification of uveitis and its relation to underlying systemic diseases.

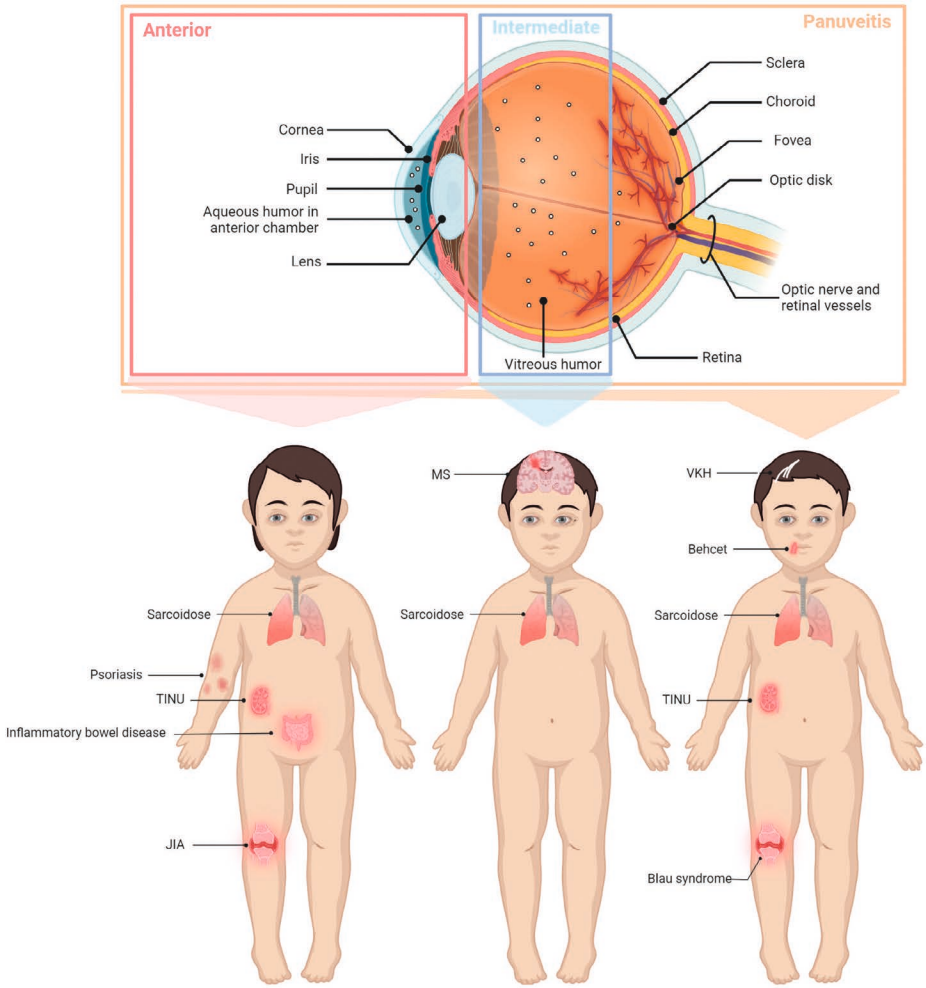



**Figure 1.** Illustration of the anatomy of the eye and the anatomical classification of uveitis and its relation to systemic inflammatory diseases.

Abbreviations: TINU, Tubulointerstitial nephritis and uveitis; JIA, juvenile idiopathic arthritis; MS, multiple sclerosis; VKH, Vogt-Koyanagi-Harada syndrome.

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## Clinical course, prognosis and impact of childhood non-infectious uveitis

Uveitis in children is in many cases a chronic condition. In contrast to adults, it is typically characterized by an asymptomatic onset (also referred to as an "insidious disease course") that often is noticed by patients after severe complications have developed. This hampers early diagnosis and it is not unusual to detect vision-threatening complications at the first visit. In fact, one third of patients have at least one complication at diagnosis. And eventually, more than three quarters develop a complication during follow-up.[8,9] The risk of development for specific complications can differ per uveitis subtype and/or systemic association. The most frequent complications seen in childhood uveitis in general are shown in **Figure 2**.[8-10] Importantly, these complications are responsible for a large proportion of blind eyes (visual acuity ≤0.1). Unilateral blindness occurs in 17-33% of patients.[8,11]

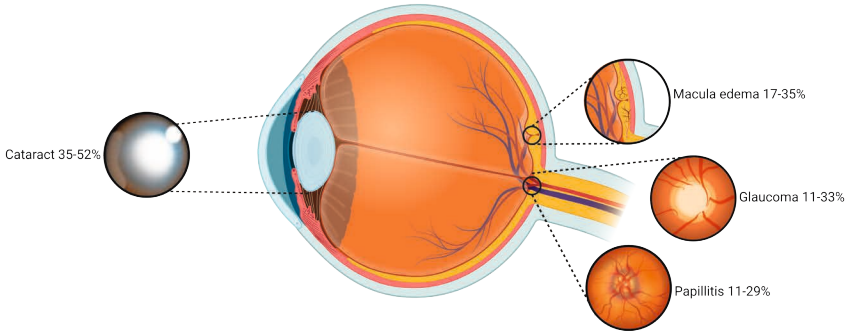


**Figure 2.** An illustration of the eye with the most frequent complications seen in different forms of childhood uveitis.

Cataract: opafication of the lens, Macula edema: cyst-like areas of fluid in the macula, glaucoma: damage of the optic nerve related to increased intraocular pressure, papillitis: optic disc swelling. Illustration created with BioRender.com.

The high risk for complications significantly impacts the psychosocial health and the well-being of children and their families.[12] This impact can be measured using a scientific metric termed 'quality of life'.[13,14] The *World Health Organization* defines *quality of life* as "an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns". Patient reported outcomes measurements to assesses the disease burden are essential in establishing personalized disease management. Previous studies have shown that children with JIA-uveitis experience a significantly

lower health-related quality of life (HRQoL) and an even worse vision-related quality of life, underscoring the detrimental impact of pediatric uveitis.[15-18] The HRQoL across the population of childhood uveitis unrelated to JIA was not investigated, but is required to determine the effect of uveitis on the child's daily participation. In addition, a frequent problem that is often associated with a lower HRQoL, is fatigue. Fatigue is a common distressing symptom in other childhood chronic auto-immune diseases.[19] More knowledge of fatigue is of great importance because it has a significant impact on the child's daily participation, including increased school absence and decreased physical functioning.[19] Early detection, timely intervention and specific attention for prevention may improve the patients' well-being and might prevent the persistence of chronic fatigue into adulthood. The prevalence and extent of fatigue in children with uveitis without systemic diseases is currently unknown.



## Treatment of children with non-infectious uveitis

Optimal management of pediatric uveitis is warranted to ensure high-quality and prompt interventions to preserve vision and improve natural development of young children into adulthood. Here, it is crucial to work in highly specialized multidisciplinary teams of uveitis-specialists, ophthalmologists and pediatric rheumatologists. The major aim of this collaborative care is to determine for each patient the optimal treatment regime that achieves maximal control of eye inflammation without inducing undesired effects of therapies that interfere with the child's well-being and development. Until recently, treatment guidelines in children with uveitis were based on expert opinions due to lack of clinical trials, which resulted in extensive variation in clinical practice. New insights and the introduction of new beneficial agents (e.g. biologics) has ensured that different international initiatives of ophthalmologists and pediatric rheumatologists originated in *Single Hub and Access point for Pediatric Rheumatology in Europe (SHARE) and The American College of Rheumatology and the Arthritis Foundation,* to develop new treatment guidelines.[20,21] These guidelines have been widely adopted and are based on so-called *step-up* approach, which refers to a sequential treatment strategy that often begins with less severe medications and shifts to more severe medications with potential side effects for patients with refractory uveitis. The treatment algorithm is demonstrated in **Figure 3.**

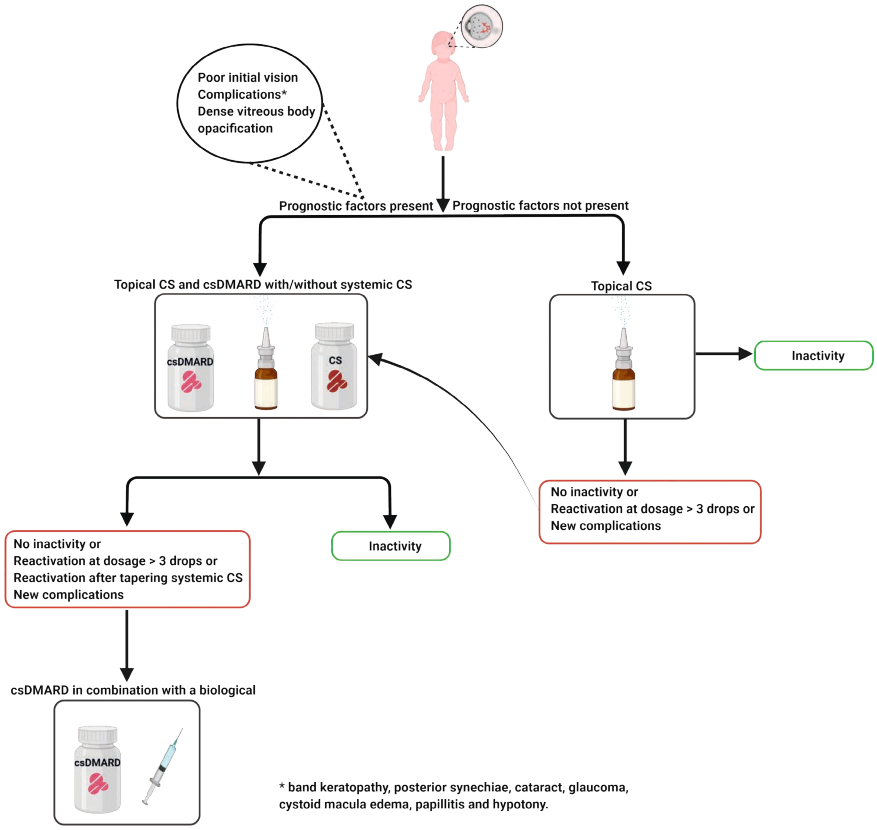


**Figure 3.** Current treatment workflow for children with non-infectious uveitis
Illustration created with BioRender.com. Abbreviations: csDMARDS, conventional synthetic disease modifying antirheumatic drugs; CS, corticosteroids.

### First-line treatment

Topical steroids are still the first-line treatment in acute and chronic uveitis.[20] However, children are frequently treated with topical corticosteroids over extended periods, which increases the risk of cataract formation and secondary glaucoma [22-24] That is, patients treated with high doses of topical corticosteroids (>3 drops daily) have a high risk of developing cataracts.[22] Systemic corticosteroids are usually not preferred due to severe side effects such as growth suppression and osteopenia.[25] However, they are potentially helpful in rapid control of severe uveitis or in the presence of cystoid macular edema (i.e. cyst-like areas of fluid in the macula), a common cause of visual loss.

**Second-line treatment**

Although the use of corticosteroids remain the mainstay for treatment of severe uveitis not related to JIA, corticosteroid-sparing immunomodulatory therapy are considered to be long-term treatment. Immunomodulatory therapy (IMT), for example conventional synthetic disease modifying antirheumatic drugs (csDMARDS) like methotrexate (MTX) and mycophenolate mofetil, are frequently initiated earlier in the disease course. These treatment agents are recommended in patients:



1) Where topical corticosteroids are insufficient or more than three corticosteroids drops daily are required for more than three months that treatment risks outweigh the beneficial effects

   or

2) If poor prognostics factors are present at the initial visit. These factors are uveitis before arthritis, posterior synechiae, male gender, band keratopathy, glaucoma, cataract, poor initial vision, hypotony, cystoid macula edema and dense vitreous body opacification.[20]

Immunomodulatory treatment is recommended so that topical corticosteroids can be gradually tapered. In fact, earlier treatment with IMT is associated with a delay of cataract development and beneficial disease outcomes.[26-28] However, in approximately a third of patients the uveitis is refractory to csDMARDs.[29] The recent SYCAMORE trial provided strong evidence that combing a csDMARD with Adalimumab (a recombinant human anti-tumor necrosis factor [TNF]-α) is beneficial in controlling eye inflammation in patients with JIA-uveitis.[30] Combination therapy with csDMARD is recommended because anti-adalimumab antibodies develop in 9% of non-JIA uveitis patients but in JIA this rises to 30%.[31,32] In patients where anti-TNF- α therapy is not sufficient, alternative biologic agents including tocilizumab, rituximab, and abatacept have shown to be effective in small number of patients with JIA-uveitis.[33-37]

Although anti-TNF-α treatment has been a major improvement in the management of children with JIA-uveitis, precautions are needed in patients with non-anterior uveitis since anti-TNF-α can cause or exacerbate multiple sclerosis.[38] Therefore, screening for white matter abnormalities by MRI is recommended before commencing therapy

in this group of patients.[31] Pre-existent asymptomatic white matter abnormalities and/or demyelination in the brain on MRI are present in 20% of patients with non-anterior uveitis.[31] So there is an unmet need for alternative, effective and safe treatment options in children with refractory non-anterior uveitis.

It is currently not possible to objectively predict the optimal treatment at the individual level. With the current treatment guidelines, it may take a considerable amount of time for a group of patients to receive their optimal therapeutic regimen. Therefore, clinical and biological driven decision tools to predict the best treatment is needed.

## Pathogenesis

**Role of immune cells in childhood non-infectious uveitis**

The pathogenesis of pediatric non-infectious uveitis remains poorly understood. Based on the clinical beneficial effects of IMT it is considered to be immune mediated, but also multifactorial given the complex genetic predisposition and association with less defined and uncertain environmental factors (e.g., post-infection, vitamin D deficiency).[39] It is hypothesized that both innate (i.e. the antigen-presenting cells dendritic cells or monocytes/macrophages) and adaptive immune response (T and B-lymphocytes) are involved in the induction of autoimmune diseases, including uveitis.[39-41] Of all immune cells, subsets of CD4+ T-lymphocytes (i.e. T helper 1 [Th1], Th2, Th17 and T regulator cells) are the most well studied, and are considered to play an important role in the pathogenesis.[39,40] In contrast, several studies support a role for B cells in the pathogenesis, mainly in JIA-uveitis. Immunohistochemical studies from iris biopsies and enucleated eyes revealed infiltrating plasma cells and CD20-positive B cells.[42] The contribution of B cells in the pathophysiology is further supported by the observation that anti-CD20 monoclonal antibody therapy (Rituximab) is effective in treating patients with (severe) JIA-uveitis.[35,36] The association of antinuclear antibodies with uveitis in JIA also supports that B cell hyperactivity contributes to the development of uveitis in patients with JIA.[43,44] However, the pathogenesis of pediatric is still poorly understood and studies have been mainly done in JIA-uveitis patients.

**HLA associations in childhood non-infectious uveitis**

Genetic studies in non-infectious uveitis, as with chronic inflammatory conditions in general, most commonly show associations with the *major histocompatibility complex* (MHC) region on chromosome 6 which encodes various *human-leukocyte antigens*

(HLA). HLA molecules are critically involved in the adaptive immunity by presenting protein fragments sampled from the cellular (class I HLA molecules) and extracellular environment (class II HLA molecules) to effectors cells (i.e., T and Natural Killer cells) eliciting an immune response.[45] HLA genes are highly polymorphic, there are >12000 HLA variants reported in humans. So that individuals possess a rich combination of HLA variants.[46] Since many variants of HLA show distinct peptide-binding specificities, it is tempting to speculate that HLA variants more often seen in patients with uveitis contribute to disease by presenting a unique (set of) autoantigen(s).



Our research group previously mapped the primary genetic risk for uveitis in JIA to the *HLA-DRB1* gene. The amino acid positions 10-12 ('*YST motif*') encoded by the *HLA–DRB1* gene exhibited a disease risk in females which was independent from the known genetic association of JIA with other amino acid positions in *HLA-DRB1.* Indeed, computational modeling revealed an altered peptide binding preference for the risk alleles that harbor the YST motif, supporting that these alleles may confer risk for uveitis by altered (autoantigen) peptide presentation to T-cells.[47] These findings also explained previous genetic associations to various *HLA-DRB1* alleles (*HLA-DRB1*03, *08, *11, *12, 13* and **14*) in JIA-uveitis patients, by demonstrating that these alleles all share the YST-motif.[48]

Also other subtypes of uveitis have been linked to *HLA* alleles in small cohort studies such as tubulointerstitial nephritis and uveitis (TINU) syndrome (*DRB1*01:02,-DQB1*05, DRB1*08:01* and *DRB1*14:01),* IU (*DRB1*15:01*) and Vogt-Koyanagi-Harada syndrome (*DRB1*04:05*).[49-56] Yet, *HLA* typing studies across a representative sample of pediatric uveitis population are sparse, but warranted because it facilitates diagnostic tools for uveitis classification.

## Immune-profiling of childhood non-infectious uveitis

The complex biological mechanisms associated with non-infectious uveitis requires novel high-throughput technologies to capture the biological fingerprint of patients and to unravel more accurately the complex pathophysiological features. Analyzing the biological fingerprint (including DNA, RNA and proteome) in combination with computational modelling may have the power to identify cryptic disease pathways that drive uveitis susceptibility. This rich information can then be exploited to develop more parsimonious models for diagnostic tools for early detection of

uveitis and future prevention tools. In addition, clinical data can be combined to identify prognostic factors. In this thesis we used different techniques to facilitate the biological fingerprint of children with uveitis (**Figure 4**).

**Next generation sequencing**

Common variations in the DNA sequence can affect biological phenotypes and consequently lead to the development of a disease. Next generation sequencing (NGS) is an advanced method that enables rapid and deep sequencing of genomes. The efficient and wide application of NGS has revolutionized our understanding of complex traits and diseases by revealing the fast landscape of genetic variations and their disease associations. Next-generation sequencing generates masses of DNA sequencing data, and is both less expensive and less time-consuming than traditional Sanger sequencing.[57] (**Figure 4**).

**RNA sequencing**

Transcription is the production of a messenger RNA copy of a DNA sequence. The *transcriptome* refers to the number of messenger RNA copies which reflects the gene expression. Whole transcriptome analysis of subsets of immune cells provides information on gene expression within a cell, both coding and non-coding genes. Since genes are thought to work together in pathways co-expression network analysis, Weighted Gene Co-expression Network Analysis (WGCNA) can be constructed to identify clusters of co-expressed genes (i.e. pathways) linked to disease. The workflow of RNA sequencing is described in **Figure 4**.

**Proteomics**

Proteomics refers to the detection and quantification of arrays of proteins in clinical samples (e.g., blood) and is the ultimate target for biomarker discovery. The blood proteome can be addressed by mass spectrometry (MS) and targeted approaches such as proximity extension assay (PEA) and Luminex assay. The inherent challenge of MS is accuracy and reproducibility in quantification analysis, in particular for the simultaneous quantification of proteins with large differences in physiological concentrations. Targeted approaches are obviously more limited by the amount of protein measured, but multiplex immunoassays such as Olink PEA allow accurately the investigation of low abundant proteins like interleukins and chemokines, with the advantage to measure relatively more proteins in relatively less volume of sample.[58] The PEA technology is described in **Figure 4**.[59]

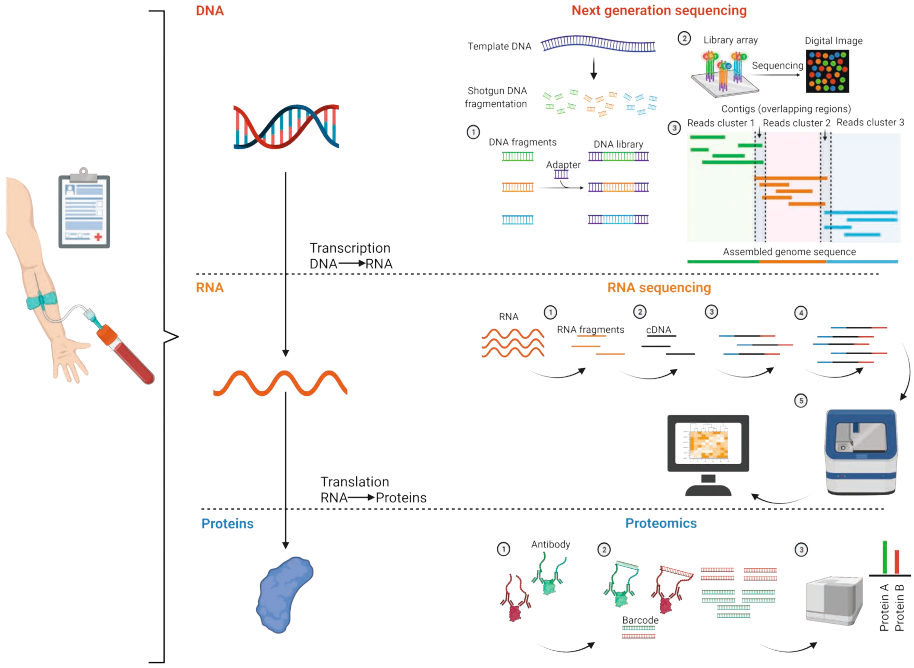


**Figure 4.** Workflow of biological profiling.

**A**) The workflow of next generation sequencing. 1) DNA is fragmented in shorter sequences (library preparation). 2) Amplification of DNA fragments (cluster generation) and fluorescently labeled nucleotides are added to the fragments. Fluorescent signal is emitted using a light source. 3) The reads are aligned to a references sequence. **B**) The workflow of RNA sequencing. 1) The RNA is splinted into small fragments. 2) The RNA fragments are converted into double stranded DNA. 3) The sequencing adapters are added to the fragments. 4) Amplification of the fragments. 5) RNA sequencing. **C**) The workflow of proteomics. 1) Antibodies bind to the protein. 2) Matched antibodies hybridize and are extended by DNA polymerase creating an unique barcode for each protein. 3) Next-generation sequencing generates a digital signal which converts the number of DNA hybridization events that corresponds to the protein concentration. Illustration created with BioRender.com.

## Aims and outline of this thesis

In this thesis we aimed to gain new insights in the disease burden, clinical course and the molecular aspects of non-infectious uveitis in children, and ultimately molecularly stratify patients, to facilitate tools that will enable personalized medicine. First, to better understand the disease burden we performed a quality of life study and focused specifically on fatigue, which is generally recognized as a frequent and distressing symptom in other childhood chronic auto-immune diseases (**chapter 2**). Over the past years novel treatments have been developed (i.e. anti-TNF-α therapy) and since ~ 2010 international multidisciplinary guidelines for the recommendation

of monitoring and treatment in children with JIA-uveitis became available. To be able to understand the impact of this shift on the prognosis in children with JIA-uveitis, we compared the clinical outcomes (i.e. complications and visual prognosis) in children diagnosed before and after the new guidelines (**chapter 3**). Anti-TNF-α therapy can be contra-indicated in patients with non-anterior uveitis due to a potentially increased risk of demyelinating disease. Therefore, we describe in **chapter 4** the results of tocilizumab (anti-IL6) treatment in children with refractory non-anterior uveitis. To identify patients with refractory uveitis in advance we aimed in **chapter 5** to stratify patients at risk for csDMARD failure using a proteomic profile. Next we performed transcriptome profiling of peripheral blood CD19-positive B of patients with JIA with and without uveitis (**chapter 6**). To identify the role of HLA molecules on uveitis we performed next generation sequencing of children with non-infectious uveitis (**chapter 7**). All the findings in this thesis are summarized and discussed in **chapter 8,** in addition to future perspectives.

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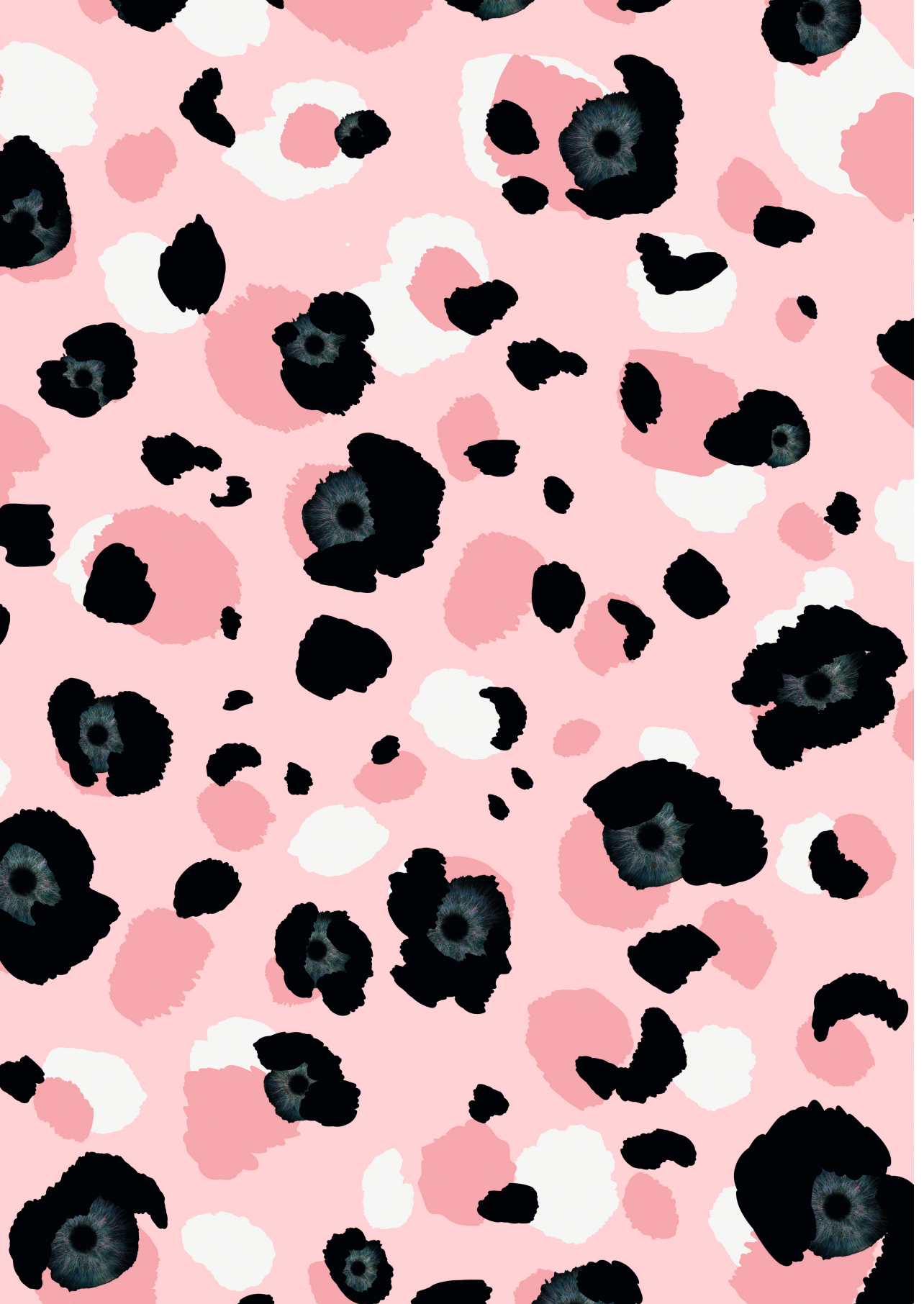

# CHAPTER 2

## Fatigue among children with non-infectious uveitis

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## ABSTRACT

**Objectives:** Knowledge on the psychosomatic burden of pediatric uveitis is lacking in current literature. Our aims were to determine the prevalence and extent of fatigue in pediatric non-infectious uveitis, and subsequently assess the relationship of fatigue on health-related quality of life (HRQoL), pain, and disease-related factors, all compared to a diseased control group and normative data of healthy peers.

**Methods:** We performed a cross-sectional study of 210 consecutive patients 2–18 years of age with juvenile idiopathic arthritis (JIA)-associated uveitis (n=34), non-JIA-uveitis (n=39), and JIA without uveitis (diseased control group, n=137). Fatigue, HRQoL, and pain were scored using the widely accepted PedsQL multidimensional fatigue scale, the PedsQL core generic score, and a visual analogue score, respectively. Clinical data on disease activity were extracted from patient charts. Regression analyses with correction for age and sex were used to compare the different groups, to calculate the effect of fatigue on HRQoL, and to identify risk factors for fatigue.

**Results**: Regardless of the severity of the uveitis, prevalence and severity of fatigue were equal between patients with uveitis, and the general population. The prevalence of severe fatigue was higher in adolescents with JIA without uveitis (13-18 years) compared to adolescents with JIA-uveitis (20% vs 0%, *P*=0.04). Fatigue was associated with a lower HRQoL (mean difference -16.9 [95% CI -25.9 to -8.0). A higher pain score was an independent risk factor for severe fatigue (odds ratio 1.4; 95% CI 1.06-1.93). Disease activity or systemic treatment did not influence severe fatigue.

**Conclusions:** In addition to the ophthalmic care of pediatric uveitis, we advise routine assessment of fatigue and pain with standardized instruments, since fatigue is associated with a lower HRQoL and pain is an independent predictor for severe fatigue. The well-being of our patients depends on more than meets the eye.

## INTRODUCTION

Non-infectious pediatric uveitis is a severe chronic inflammatory eye disease and accounts for 5-10% of all uveitis cases.[1] Uveitis can manifest as part of a systemic disease of which juvenile idiopathic arthritis (JIA) associated uveitis is the most common form.[2] With its typically asymptomatic chronic character, JIA associated uveitis (JIA-uveitis) has a high risk of ocular complications and severe visual loss.[2] Whereas other forms of uveitis can present with pain, redness of the eye or floaters.



Over the past years, the clinical outcomes of both uveitis and JIA have improved by new treatment strategies (e.g. anti-tumor necrosis factor alpha [TNFα]).[3-5] Yet, recent studies have shown that children with uveitis report a lower health-related quality of life (HRQoL) and vision-related quality of life, and this continues in adulthood with also an increased prevalence of anxiety and depression.[6,7] In addition, adult patients with JIA (regardless uveitis) who use systemic immunomodulatory treatment experience lower HRQoL.[7] A frequent problem in pediatric auto-immune diseases, that is often associated with lower HRQoL, is fatigue.[8,9] Fatigue is a common symptom among adolescents with JIA and is associated with significant impairments, including increased school absence and decreased physical functioning.[8,9] Even though fatigue is generally recognized as a frequent and distressing symptom in other childhood chronic auto-immune diseases, data on frequency and extent of fatigue in children with non-infectious uveitis, as well as its relationship with disease-related factors, is currently unknown. More knowledge of fatigue is of great importance, due to its significant impact on the child's psychosocial and school functioning.[8,9] In addition, early detection and timely intervention of fatigue may improve the patients' well-being and their participation in daily life.[10] It may even prevent the persistence of chronic fatigue into adulthood, subsequently improving long-term quality of life. Therefore, this study primarily aims to investigate the prevalence and extent of fatigue in children with non-infectious uveitis and two control groups (i.e. general population and JIA without uveitis) and subsequently assess the relationship of fatigue on health-related quality of life, pain, and disease-related factors.

## METHODS

### Patients and study design

A cross-sectional study was conducted in the outpatient clinic of the department of ophthalmology at University Medical Centre Utrecht in the Netherlands. The study was approved by the Medical Ethical Research Committee of the University Medical Centre Utrecht in concordance with the Declaration of Helsinki (Medical Research Ethics Committee Utrecht no 21-041). Children aged 2-18 years with idiopathic chronic non-infectious uveitis (i.e., non-JIA-uveitis) diagnosed before the age of 16 were eligible. Written informed consent was obtained from all patients ≥ 16 years, from both parents and patients in cases 12-16 years of age, and from parents in cases < 12 years old. Data of children with JIA with and without uveitis were obtained from a previously reported cohort.[8,9] Fatigue scores of children with juvenile idiopathic arthritis-associated uveitis (JIA-uveitis, *n*=34) and idiopathic chronic uveitis (i.e., non-JIA-uveitis [*n*=39]) were compared with data from the general population (n=298) [9,11] as well as data from children with juvenile idiopathic arthritis without uveitis (n=137).

The diagnosis of uveitis was established by a trained uveitis specialist according to the Standardization of the Uveitis Nomenclature (SUN) criteria.[12] Juvenile idiopathic arthritis was diagnosed according to the criteria of the *International League of Associations for Rheumatology*.[13] Patients with JIA were screened by an ophthalmologist according to the guidelines of the American Academy of Pediatrics.[14] Patients with SUN 1+ cells or more in the anterior chamber and/or vitreous body during ophthalmologic examinations were diagnosed with uveitis.

### Study procedure

All patients that gave consent to participate in the study were invited to register at home using a web-based tool (www.hetklikt.nu). Using this online tool, patients completed the questionnaires electronically maximum two weeks prior to their doctor's appointment. The validated (self-administered) questionnaires Pediatric Quality of Life Inventory multidimensional fatigue scale (PedsQL-MFS), Pediatric Quality of Life Inventory – core generic score (PedsQL-GCS), and visual analogue score (VAS) were used to assess fatigue, HRQoL and pain, respectively. The PedsQL-MFS comprises a general fatigue scale, sleep/rest fatigue scale and cognitive fatigue scale and yields a score ranging from 0-100. A lower score indicates more fatigue.

Severe fatigue was defined as a value of >2SD below the norm on the subscale total fatigue PedsQL-MFS.[9]

The PedsQL-GCS consists of four multidimensional scales with a score ranging from 0-100 for each domain: physical functioning, emotional functioning, social functioning, and school functioning. Psychosocial functioning was defined as a combination of emotional functioning, social functioning, and school functioning. A lower score indicates a lower HRQoL. The percentage of school absence was calculated over the past six months. For patients aged 2-7 years old, only parents filled out the questionnaires. For patients aged 8-18 years old, both children and parents filled out the questionnaires. In five cases questionnaires were parent-proxy reported instead of child reported.



**Clinical outcomes**

For all participants the clinical records were reviewed in order to collect information about sex, age, type of uveitis, type of JIA, disease status, ocular comorbidities, and systemic therapy. Disease activity was scored according to the SUN criteria and the clinical Juvenile Arthritis Disease Activity Score-27 (cJADAS-27) for uveitis and JIA respectively. The SUN criteria are based on the total number of cells in the anterior chamber and/or vitreous body so that a higher score corresponds to more disease activity.[12] The cJADAS-27 for JIA is based on four domains 1) physician global assessment of disease activity on a visual analogue scale from 0-10 2) parent/patient global assessment of disease activity on a visual analogue scale from 0-10 3) active joint count (swelling of the joints) of in total 27 specified joints 4) normalized erythrocyte sedimentation rate (ESR) count on a scale of 0-10.[15] A higher score indicates more disease activity and the maximum score is 57.

**Statistical analyses**

Statistical analyses of the data were performed using R software version 3.6.1. Pearson's $\chi 2$ test or Fisher's exact test was used for categorical variables, and a Student's t-test, Mann-Whitney U test or Kruskal-Wallis test for continuous variables. The different patient groups stratified per age group were compared with healthy peers using previously published data on a reference population of the same age (i.e. 8-12 years and 13-18 years).[9,11] We used linear regression analyses, with correction for age and sex, to see whether patient groups (non-JIA-uveitis versus JIA-uveitis and JIA without uveitis versus JIA-uveitis) affected fatigue. The differences are reported as

mean difference (unstandardized beta) with a 95% confidence interval (CI). In addition, linear regression was used in order to assess the effect of severe fatigue on HRQoL and school absence. Logistic regression was applied in order to assess predictors for severe fatigue. The following potential predictors were analyzed in the univariate model: sex, age, type of uveitis, duration of uveitis, uveitis activity, VAS pain score, systemic treatment, and ocular complications. In the multivariate analysis we entered the variables with $P < 0.05$ in the univariate analysis and we additionally adjusted for sex and age. P-values of less than 0.05 and a confidence interval that did not contain a zero were considered to be significant. All tests were 2- tailed.

## RESULTS

### Study population

In total 210 patients ranging from 2 to 18 years were included in this study of which 39 patients were diagnosed with non-JIA-uveitis, 34 patients with JIA-uveitis and 137 patients were diagnosed with JIA without uveitis. Two patients were aged between 2-7 years, 81 patients were aged between 8-12 years and 127 patients were aged between 13-18 years. In the groups 8-12 years and 13-18 years, respectively, three and two patients did not completed the child reported questionnaires and therefore the parent-proxy-reported questionnaires were used to assess fatigue, HRQoL and pain. Baseline characteristics of the patients are presented in **Table 1**. Patients with JIA without uveitis had a shorter duration of arthritis and a higher JADAS-27 score compared to patients with JIA-uveitis (**Table 1**). As expected, the non-JIA-uveitis group contained significantly more males compared to patients with JIA with and without uveitis (54%, 29% and 32%, respectively). In addition, more patients with non-JIA-uveitis were treated with systemic corticosteroids. Patients with uveitis were more frequently treated with a DMARD compared to JIA patients without uveitis. There was no difference in ocular complications between the uveitis groups.

**Table 1.** Demographic characteristics of patients with juvenile idiopathic arthritis associated uveitis, non-juvenile idiopathic arthritis associated uveitis and juvenile idiopathic arthritis without uveitis.

| | Total uveitis (n=73) | Non-JIA-uveitis (n=39) | JIA-uveitis (n=34) | JIA without uveitis (n=137) | *P-value* [a] |
|---|---|---|---|---|---|
| Male, n (%) | 31 (42) | 21 (54) | 10 (29) | 44 (32) | 0.03 |
| Age, median in years (IQR) | 13 (11-15) | 14 (12-15) | 13 (10-16) | 14 (12-17) | 0.19 |
| Duration of arthritis, median in years (IQR) | N.A. | N.A. | 8 (5-10) | 6 (3-9) | 0.03 |
| Duration of uveitis, median in years (IQR) | 4.5 (2.0-7.4) | 3.3 (1.4-6.1) | 6.7 (3.2-9.3) | N.A. | 0.01 |
| Arthritis activity (cJADAS-27), n (%) | 0 (0-1.0) | N.A. | 0 (0-1.0) | 1 (0-4) | 0.04 |
| Uveitis activity, n (%) | 18 (22) | 10 (26) | 8 (26) | N.A. | 0.99 |
| Systemic corticosteroids, n (%) | 11 (15) | 10 (26) | 1 (3) | 3 (2) | $1.86 \times 10^{-5}$ |
| DMARD, n (%) | 64 (88) | 34 (87) | 30 (88) | 56 (41) | $5.64 \times 10^{-10}$ |
| Anti-TNF-α therapy, n (%) | 32 (44) | 13 (33) | 19 (56) | 45 (33) | 0.04 |
| Cataract surgery, n (%) | 15 (21) | 10 (26) | 5 (16) | 0 (0) | 0.39 |
| Secondary glaucoma, n (%) | 9 (12) | 4 (10) | 5 (16) | 0 (0) | 0.50 |
| BCVA in decimals, median (IQR) [b] | 1.0 (0.7-1.2) | 1.0 (0.7-1.2) | 1.0 (0.9-1.2) | N.A. | 0.12 |
| Visual impairment, n (%) [c] | 3 (4) | 2 (5) | 1 (3) | 0 (0) | 1.00 |

[a] non-JIA-uveitis vs JIA-uveitis vs JIA uveitis without uveitis.
[b] Best-correct visual acuity of worst eye.
[c] Visual impairment was defined as BCVA between 0.4 and 0.1 in decimals.
IQR, Interquartile range; N.A., not applicable; cJADAS-27, clinical Juvenile Arthritis Disease Activity Score-27; DMARD, disease modifying antirheumatic drugs; TNF- α, tumor necrosis factor alpha; BCVA, best-corrected visual acuity.

**The prevalence of severe fatigue and mean fatigue score between children and adolescents with uveitis and the general population**

The prevalence and the fatigue scores per age category for children and adolescents with uveitis and the general reference population are presented in **Figure 1A, Figure 1B** and **Supplementary table 1.** There was no significant difference in the prevalence of severe fatigue (defined as >2SD on the subscale total PedsQL-MFS) between uveitis patients and the general population for both age groups (3.4% vs 3.9% and 9.5% versus 6.7% for respectively, 8-12 years and 13-18 years). There was also no significant difference in mean fatigue scores between patients and the general population for both age groups.

**The prevalence of severe fatigue and mean fatigue score between the different patient groups**

There was no significant difference in the prevalence of severe fatigue between patients with JIA-uveitis and with non-JIA-uveitis for both age groups (**Supplementary table 1**). Linear regression analyses, with correction for age and sex, revealed that having a history of JIA in patients with uveitis did not influence the total fatigue score (2.1; 95% CI -5.2 to 9.4) (**Table 2**).

The prevalence of severe fatigue was higher in adolescents with JIA without uveitis (13-18 years) compared to adolescents with JIA-uveitis (20% vs 0%, $P$ =0.04). A history of uveitis in patients with JIA did not influence the total fatigue score (4.7; 95% CI -2.3 to 11.8) (**Table 2**).

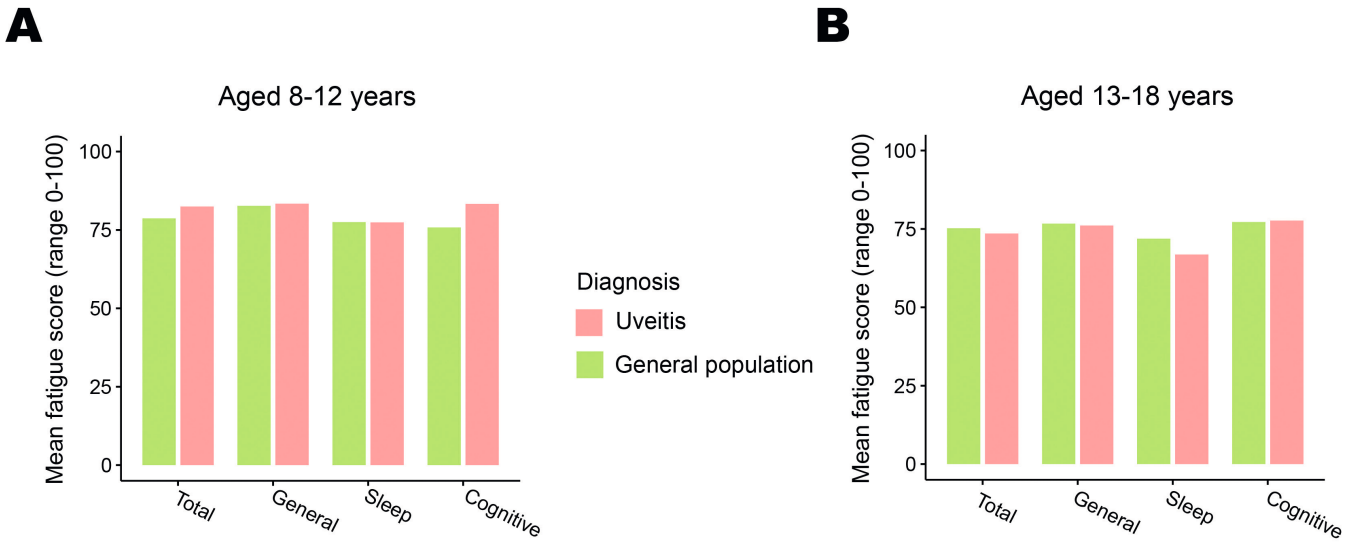


**Figure 1.** Mean fatigue scores (PedsQL -MFS) in patients with uveitis and general population per age group.[9]

The lower the score the more fatigue. **A)** Total mean fatigue scores and the specific domains "general", "sleep" and "cognitive" in uveitis and general population aged 8-12 years. **B)** Total mean fatigue scores and the specific domains "general", "sleep" and "cognitive" in uveitis and general population aged 13-18 years.

**Table 2.** Unadjusted and adjusted mean differences in fatigue scores between children and adolescents with non-juvenile idiopathic arthritis associated uveitis (n=39), juvenile idiopathic arthritis associated uveitis (n=34), and juvenile idiopathic arthritis without uveitis (n=137).

| | Non-JIA uveitis vs JIA-uveitis | | | | JIA without uveitis vs JIA-uveitis | | | |
|---|---|---|---|---|---|---|---|---|
| | Unadjusted model | | Adjusted model | | Unadjusted model | | Adjusted model | |
| | Mean difference | 95% CI | Mean difference | 95% CI | Mean difference | 95% CI | Mean difference | 95% CI |
| PedsQL-MFS-Total | 1.7 | -5.5 to 8.9 | 2.1 | -5.2 to 9.4 | 5.9 | -1.4 to 13.1 | 4.7 | -2.3 to 11.8 |
| PedsQL-MFS-General | -6.1 | -14.6 to 2.5 | -6.1 | -14.9 to 2.7 | 3.2 | -5.7 to 12.1 | 1.3 | -7.2 to 9.8 |
| PedsQL-MFS-Sleep | 3.2 | -6.2 to 12.6 | 2.6 | -6.7 to 12.0 | 4.8 | -2.6 to 12.1 | 2.8 | -4.2 to 9.9 |
| PedsQL-MFS-Cognitive | 5.6 | -3.0 to 14.3 | 6.4 | -2.7 to 15.4 | 7.2 | -1.6 to 16.1 | 7.2 | -1.8 to 16.2 |

The adjusted model was corrected for age and sex. The PEDsQL-MFS is scored on a scale from 0 to 100, with a lower score indicating more fatigue. Thus, a negative mean difference indicates a lower score, indicating more fatigue in JIA-uveitis.
PedsQL MFS, PedsQL multidimensional fatigue scale, CI, confidence interval.

**Severe fatigue and the association with a lower health-related quality of life score**

There was no difference in the overall HRQoL between uveitis patients and the general reference population for both children and adolescents **(Supplementary table 2)**. However, patients with uveitis aged between 13-18 years had a significantly lower HRQoL on the domains physical functioning (85.3±14.6 vs. 90.7±13.4, *P* = 0.02) and school functioning (71.2±15.8 vs. 78.1±17.8, *P* = 0.01). Patients with uveitis reported significantly higher school absence in the past six months than healthy controls (8.2%±9.7 versus 4.9%±6.9, *P* = 0.04, data not shown).[8] Severe fatigue was significantly associated with a lower HRQoL score (**Table 3**). This significant association was found with respect to all domains, except for social functioning and school absence.



**Table 3.** The effect of severe fatigue on health-related quality of life and school absence in children with uveitis (n=73).

| | Unadjusted model | | Adjusted model [a] | |
|---|---|---|---|---|
| | Mean difference | 95% CI | Mean difference | 95% CI |
| PedsQL GCS (range 0–100) *2–18 years of age* | | | | |
| Total functioning | -17.6 | -26.4 to -8.8 | -16.9 | -25.9 to -8.0 |
| Physical functioning | -13.7 | -25.3 t -2.2 | -12.3 | -24.0 to -0.7 |
| Emotional functioning | -25.1 | -38.4 to -11.9 | -24.4 | -37.9 to -10.9 |
| Social functioning | -8.4 | -19.4 to 2.5 | -8.5 | -19.7 to 2.8 |
| School functioning | -26.2 | -38.1 to -14.2 | -25.7 | -37.7 to -13.7 |
| Psychosocial functioning | -19.9 | -30.0 to -9.9 | -19.4 | -29.7 to -9.2 |
| School absence | 10.0 | 1.1 to 18.9 | 8.3 | -1.4 to 18.1 |

[a] The adjusted model was corrected for age and sex. PedsQL-GCS is scored on a scale from 0 to 100, with lower scores indicating lower health-related quality of life. Severe fatigue was dichotomized as above (1) or below (0) the cut off score for severe fatigue (>2SD below the norm on the subscale total fatigue PedsQL-MFS).[9] Having severe fatigue decreased the health-related quality of life. PedsQL-GCS, PedsQL generic core scale, CI, confidence interval.

**Disease-related factors and severe fatigue**

Characteristics of severe fatigued and non-severe fatigued patients are presented in **Table 4**. Multivariate analyses, with correction for age and sex, showed that a higher VAS pain score was an independent risk factor for severe fatigue (odds ratio 1.4; 95% CI 1.06 to 1.93). Treatment with systemic corticosteroids and immunomodulatory treatment did not influence fatigue.

**Table 4.** Characteristics of severe fatigue (n=6) and non-severe fatigue (67) uveitis patients.

| | Severe fatigue (n=6) | Non-severe fatigue (n=67) | *P-value* |
|---|---|---|---|
| Male, n (%) | 1 (17) | 30 (45) | 0.23 |
| Age, median in years (IQR) | 15 (13-16) | 13 (11-15) | 0.49 |
| JIA-uveitis, n (%) | 1 (17) | 33 (49) | 0.21 |
| Duration of uveitis, median in years (IQR) | 3.6 (0.4-7.6) | 4.5 (2.1-7.3) | 0.37 |
| Uveitis activity, n (%) | 2 (33) | 16 (67) | 0.64 |
| VAS pain score (0-10), median | 6.0 (2.0-7.8) | 1.0 (0.0-3.0) | 0.04 |
| Systemic corticosteroids, n (%) | 2 (33) | 9 (13) | 0.22 |
| DMARD, n (%) | 5 (83) | 59 (88) | 0.56 |
| Anti-TNF-α therapy, n (%) | 1 (17) | 31 (46) | 0.22 |
| Ocular complications, n (%) [a] | 1 (17) | 20 (30) | 0.67 |
| Visual impairment, n (%) [b] | 0 (0) | 3 (5) | 1.0 |

[a] Complications: cataract surgery and/or secondary glaucoma.
[b] Visual impairment was defined as BCVA between 0.4 and 0.1 in decimals. IQR, Interquartile range; VAS, visual analogue scale; DMARD, disease modifying antirheumatic drugs; TNF- α, tumor necrosis factor alpha; BCVA, best-corrected visual acuity.

## DISCUSSION

Our study shows that regardless of the severity of the uveitis, there was no difference in prevalence and severity of fatigue between patients with uveitis and the general population. However and more importantly, if patients with uveitis have severe fatigue they do report a lower health-related quality of life. Lastly, not disease activity or systemic treatment but a higher pain score influenced severe fatigue. The results of this study emphasize the need for routinely assessment of fatigue and pain in addition to ophthalmic care to maintain or improve the well-being of children with uveitis.

Although fatigue is more prevalent among children and adolescents with different chronic diseases and can be seen as a non-disease specific symptom, we did not detect a significant difference between uveitis patients and the general population.[9,16,17] Indeed, our finding is consistent with a recent paper that report a normal HRQoL and fatigue in children with non-infectious uveitis.[18] Also, having a history of uveitis did not influence the total fatigue score in patients with JIA. In fact, the prevalence of severe fatigue in adolescents (13-18 years) with JIA-uveitis was lower compared to adolescents with JIA without uveitis. A possible explanation for this is that fatigue is multidimensional. So to say, biological / lifestyle (i.e. physical

functioning), psychological (e.g. anxiety or depression) and social factors (e.g. pressure at school) are strongly associated with fatigue. In fact, almost three-quarters of the variance in fatigue could be explained by these non-disease-specific biopsychosocial factors in patients with a chronic autoimmune disease, cystic fibrosis (CF), or post-cancer treatment.[17] One can speculate that CF and post-cancer treatment patients may experience a larger impact of their disease on their lifestyle and psychosocial functioning due to their disease than children with uveitis and may therefore be more at risk for fatigue. For example, children with uveitis do not report a clinically relevant score on the revised child anxiety and depression scale.[6] However, future studies should focus on the relationship between uveitis and impact on fatigue or well-being with consideration of both biological and psychosocial factors.



Severe fatigue was associated with a lower HRQoL. Furthermore, a higher pain score was an independent risk factor for severe fatigue. Five out of six patients with severe fatigue were diagnosed with non-JIA-uveitis. This indicates that pain is not exclusive to JIA but also exists in uveitis not associated with JIA which is also reported in adult patients with non-anterior uveitis.[19] Disease activity or treatment with systemic corticosteroids or immunomodulatory agents did not influence severe fatigue. This is in line with other studies suggesting that disease-specific factors do not play a large role in explaining fatigue in a stable phase of the disease. [8,16,17,20] So, despite the fact that children with uveitis frequently require systemic corticosteroids and/or immunomodulatory therapy, which reflects the severity of the inflammation, this seems to have little impact on the well-being. It is important to know for doctors as well as patients and parents as initiating immunomodulatory therapy earlier in the disease course is recommended.

Based on our study and a previous study, there is no increased fatigue and decreased overall HRQoL in children with uveitis.[18] However, these studies may underestimate the impact of the disease on the child since they are lacking disease specific limitations for example those related to vision.[21] Therefore, the vision-related questionnaire The Effects of Youngsters' Eyesight on Quality of Life questionnaire (EYE-Q) is currently being developed for other languages to assess visual outcomes and visual-related quality of life in children with uveitis.[22] Hence, future studies should be focusing on the visual-related quality of life and with special focus on the relationship with developmental stage.

The percentage of school absence was significantly higher in patients with uveitis. More fatigue can potentially be associated with higher school absence.[8] However, we did not see this association in our study. This suggests that other factors also influence school absence in children with uveitis, e.g. the frequent hospital visits for both the ophthalmologists and rheumatologists. The underlying causes need to be specifically explored in further studies.

One of the limitations of our study is that in five cases, we used parent-reported data in our analysis. Parent's reporting of their child can differ from the child's own reporting.[23,24] However, we do not consider it likely that this influenced our results as we did not observe any differences between the groups. Secondly, the sample size of our study was rather small and therefore we may be less likely to detect small differences.

In conclusion, to maintain or improve the well-being and development of young children we advise to perform routine assessment of fatigue and pain in the consultation room since fatigue is associated with a lower quality of life, and pain is an independent predictor for severe fatigue. Disease-specific factors or systemic treatment did not influence severe fatigue.

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## SUPPLEMENTARY DATA

**Supplementary table 1.** Fatigue scores per age category for children and adolescents with uveitis (n=71), juvenile idiopathic arthritis associated uveitis (n=33), non-juvenile idiopathic arthritis associated uveitis (n=38), juvenile idiopathic arthritis without uveitis (n=137) and general population per age.

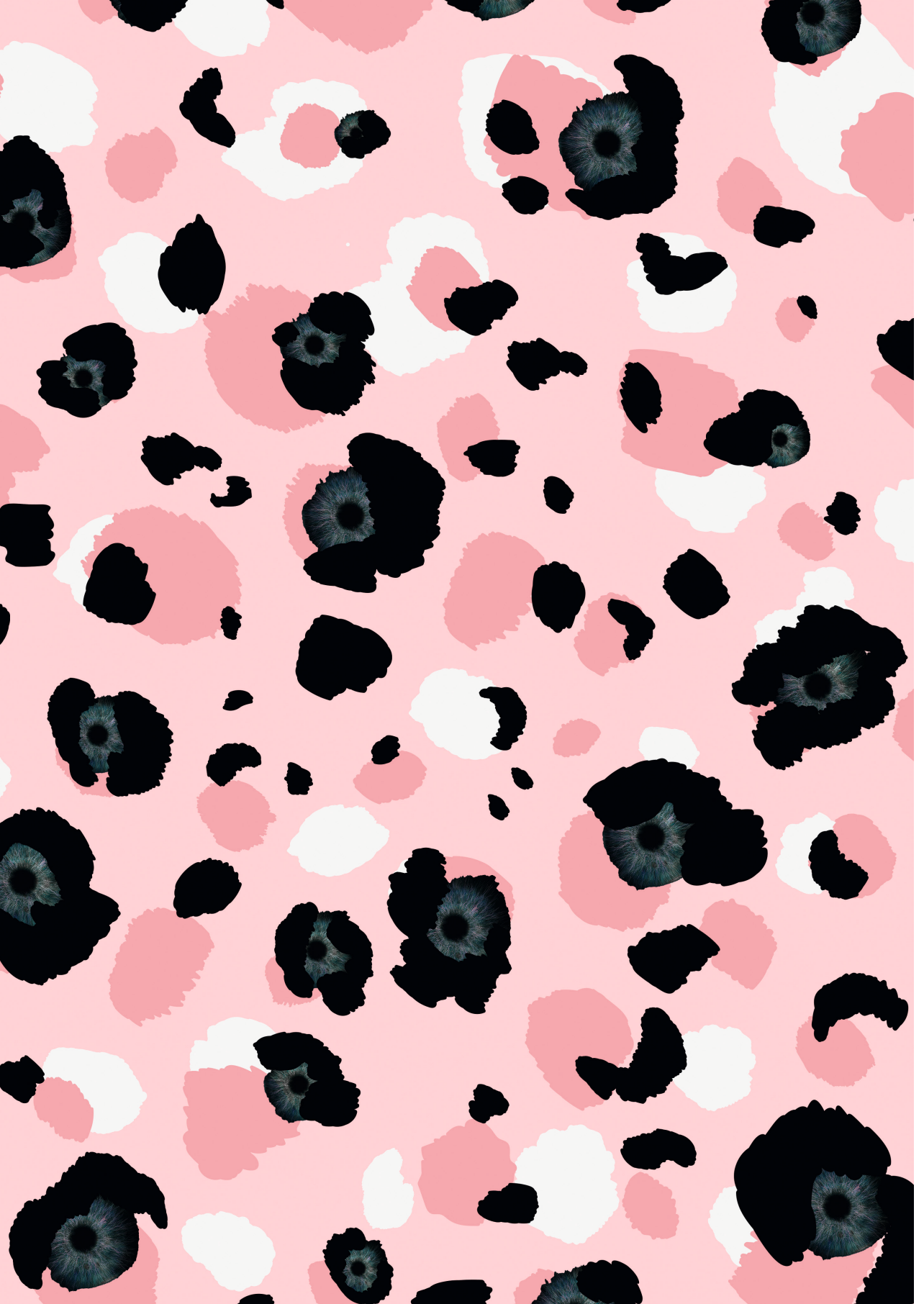
| | Total uveitis | | JIA-uveitis | | Non-JIA-uveitis | | JIA | | Reference[9] |
|---|---|---|---|---|---|---|---|---|---|
| **Child report 8–12 years old**[a] | n=29 | | n=15 | | n=14 | | n=52 | | n=143 |
| PedsQL-MFS | Median (IQR) | Mean ± SD | Median (IQR) | Mean ± SD | Median (IQR) | Mean ± SD | Median (IQR) | Mean ± SD | Mean ± SD |
| PedsQL-MFS-Total | 85.4 (76.0-93.4) | 82.5±13.8 | 83.3 (73.3-94.1) | 80.6±16.3 | 85.4 (77.7-91.6) | 84.4±11.0 | 78.5 (70.8-86.5) | 77.5±14.8 | 78.7±12.5 |
| PedsQL-MFS-General | 91.6 (70.8-100) | 83.4±19.6 | 70.8 (68.7-97.9) | 76.2±22.4 | 97.9 (84.3-100) | 91.0±12.9 | 83.3 (70.8-95.8) | 80.1±18.9 | 82.7±12.9 |
| PedsQL-MFS-Sleep | 79.1 (66.7-95.8) | 77.4±18.3 | 79.1 (64.6-93.7) | 76.1±20.4 | 79.1 (71.8-94.8) | 78.8±16.5 | 75.0 (66.7-83.3) | 74.8±13.5 | 77.5±15.0 |
| PedsQL-MFS-Cognitive | 87.5 (73.9-100) | 83.3±17.6 | 93.8 (69.8-100) | 83.3±20.3 | 83.3 (75.0-98.9) | 83.3±15.3 | 79.2 (66.7-95.8) | 77.5±21.2 | 75.8±19.1 |
| Severe fatigue, n (%) | 1 (3.4) | | 1 (7) | | 0 (0) | | 4 (8) | | 3.9 |
| **Child report 13–18 years old**[b] | n=42 | | n=18 | | n=24 | | n=85 | | n=155 |
| PedsQL-MFS | Median (IQR) | Mean ± SD | Median (IQR) | Mean ± SD | Median (IQR) | Mean ± SD | Median (IQR) | Mean ± SD | Mean ± SD |
| PedsQL-MFS-Total | 74.3 (62.5-73.5) | 73.5±15.1 | 77.1 (63.2-81.9) | 75.3±13.8 | 72.2 (61.1-81.2) | 72.2±16.2 | 68.1 (55.6-86.1) | 68.6±21.6 | 75.2±11.9 |
| PedsQL-MFS-General | 79.2 (68.7-70.8) | 76.1±17.0 | 79.2 (75.0-83.3) | 75.0±17.3 | 77.1 (66.6-89.6) | 76.9±17.2 | 70.8 (50.0-91.7) | 67.1±26.2 | 76.7±14.3 |
| PedsQL-MFS-Sleep | 68.7 (55.2-79.1) | 66.8±20.2 | 70.8 (56.3-79.2) | 69.2±19.1 | 66.6 (57.2-79.1) | 65.1±21.2 | 62.5 (50.0-83.3) | 63.9±21.3 | 71.9±14.2 |
| PedsQL-MFS-Cognitive | 79.1 (66.6-95.8) | 77.6±18.7 | 85.4 (71.9-95.8) | 81.7±17.8 | 72.9 (66.6-89.6) | 74.6±19.2 | 83.3 (62.5-95.8) | 74.6±25.9 | 77.2±15.3 |
| Severe fatigue, n (%) | 4 (9.5) | | 0 (0) | | 4 (17) | | 17 (20) | | 6.7 |

[a] In the group of children aged 8-12 years, three questionnaires were parent-proxy-reported instead of child reported.
[b] In the group of children aged 13-18 years, two questionnaires were parent-proxy-reported instead of child reported.
PedsQL-MFS score are presented in medians with interquartile ranges and mean with standard deviation for patients and mean with standard deviation for the reference population. PEDsQL-MFS are scored on a scale from 0 to 100, with a lower score indicating more fatigue. Data of the reference population were obtained from Nap-van der Vlist et al.[9] Severe fatigue was defined as >2SD below the norm on the subscale total fatigue PedsQL-MFS.[9]
IQR, interquartile range; N.A., not applicable. PedsQL MFS, PedsQL multidimensional fatigue scale

**Supplementary table 2.** Health-related quality of life scores per age category for children and adolescents with uveitis (n=71), juvenile idiopathic arthritis associated uveitis (n=33), non-juvenile idiopathic arthritis associated uveitis (n=38), juvenile idiopathic arthritis without uveitis (n=137) and general population per age.

| | Total uveitis | | JIA-uveitis | | Non-JIA uveitis | | JIA | | Reference[11] |
|---|---|---|---|---|---|---|---|---|---|
| Child report 8–12 years old[a] | n=29 | | n=15 | | n=14 | | n=52 | | n=475 |
| PedsQL-GCS | Median (IQR) | Mean ± SD | Median (IQR) | Mean ± SD | Median (IQR) | Mean ± SD | Median (IQR) | Mean ± SD | Mean ± SD |
| PedsQL-Total | 85.9 (78.3-94.5) | 84.5±11.7 | 85.8 (73.9-94.0) | 82.3±13.6 | 90.7 (82.5-94.2) | 86.9±9.2 | 83.7 (69.0-89.7) | 79.1±13.8 | 85.3±11.7 |
| PedsQL-Physical | 93.6 (78.1-100) | 88.5±13.0 | 87.5 (73.4-100) | 84.8±15.3 | 93.8 (90.6-100) | 92.4±9.1 | 84.4 (68.8-96.9) | 80.9±18.2 | 92.6±11.2 |
| PedsQL-Emotional | 80.0 (75.0-90.0) | 81.2±16.0 | 80.0 (75.0-92.5) | 80.0±18.3 | 87.5 (71.3-90.0) | 82.5±13.6 | 80.0 (60.0-90.0) | 74.7±17.5 | 78.2±17.1 |
| PedsQL-Social | 90.0 (85.0-100) | 87.4±14.7 | 90.0 (75.0-97.5) | 85.7±15.1 | 95.0 (86.3-100) | 89.3±14.5 | 90.0 (75.0-95.0) | 83.4±16.6 | 83.5±16.9 |
| PedsQL-School | 82.5 (73.8-90.0) | 79.8±14.7 | 82.5 (71.3-90.0) | 78.9±17.5 | 85.0 (76.3-90.0) | 80.7±11.9 | 80.0 (67.5-85.0) | 76.2±14.3 | 82.8±15.4 |
| PedsQL-Psychosocial | 85.0 (78.2-93.3) | 83.0±14.0 | 83.3 (79.1-93.3) | 82.0±16.7 | 89.1 (77.0-91.6) | 84.1±11.0 | 81.7 (68.3-86.7) | 78.1±12.9 | 81.5±13.8 |
| VAS pain score (0-10) | 1.0 (0.0-1.0) | 1.1±1.7 | 1.00 (0.0-1.50) | 1.1±1.2 | 0.00 (0.0-1.0) | 1.1±2.14 | 1.0 (0-3) | 2.1±2.6 | N.A. |
| Child report 13–18 years old[b] | n=42 | | n=18 | | n=24 | | n=85 | | n=491 |
| PedsQL-GCS | Median (IQR) | Mean ± SD | Median (IQR) | Mean ± SD | Median (IQR) | Mean ± SD | Median (IQR) | Mean ± SD | Mean ± SD |
| PedsQL-Total | 82.5 (72.0-90.1) | 81.0±11.0 | 84.2 (72.8-91.0) | 82.8±10.0 | 78.1 (71.9-88.5) | 79.7±11.8 | 75.0 (64.1-91.3) | 75.2±18.9 | 84.5±13.5 |
| PedsQL-Physical | 90.6 (81.3-96.1) | 85.3±14.6 | 87.5 (82.0-93.8) | 85.2±14.7 | 90.6 (74.2-97.7) | 85.3±14.9 | 75.0 (59.4-93.8) | 72.9±23.5 | 90.7±13.4 |
| PedsQL-Emotional | 80.0 (70.0-90.0) | 77.5±17.2 | 85.0 (70.0-95.0) | 80.8±18.2 | 75.0 (68.8-85.0) | 75.0±16.3 | 75.0 (60.0-100) | 75.9±23.0 | 80.4±19.5 |
| PedsQL-Social | 90.0 (80.0-100) | 88.0±12.0 | 92.5 (82.5-100) | 90.0±10.9 | 90.0 (80.0-96.3) | 86.5±12.7 | 90.0 (75.0-100) | 83.9±18.2 | 85.2±17.0 |
| PedsQL-School | 75.0 (65.0-80.0) | 71.2±15.8 | 75.0 (66.3-85.0) | 73.6±15.4 | 70.0 (63.8-75.0) | 69.4±16.2 | 70.0 (55.0-90.0) | 69.2±22.8 | 78.1±17.8 |
| PedsQL-Psychosocial | 80.8 (69.9-88.3) | 78.8±12.2 | 85.8 (73.8-89.6) | 81.5±11.7 | 75.7 (69.9-86.6) | 76.9±12.4 | 78.3 (68.3-91.7) | 76.4±18.2 | 81.2±15.5 |
| VAS pain score (0-10) | 1.0 (0.0-5.0) | 2.8±3.0 | 2.0 (0.25-6.0) | 3.3±3.2 | 1.0 (0.0-3) | 2±2.7 | 2.0 (1.0-6.0) | 3.0±2.8 | N.A. |

[a] In the group of children aged 8-12 years three questionnaires were parent-proxy-reported instead of child reported.
[b] In the group of children aged 13-18 years, two questionnaires were parent-proxy-reported instead of child reported.
PedsQL-GCS score are presented in medians with interquartile ranges and mean with standard deviation for patients and mean with standard deviation for the reference population. PedsQL-GCS is scored on a scale from 0 to 100, with lower scores indicating lower health-related quality of life. Data of the reference population were obtained from van Muilekom et al.[11]
IQR, interquartile range; VAS, visual analogue scale; N.A.; not applicable. PedsQL-GCS, PedsQL generic core scale.



# CHAPTER 3

## Improved clinical outcomes in children with JIA associated uveitis in the last decade

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## ABSTRACT

**Purpose:** To analyze the development of ocular complications and visual prognosis in juvenile idiopathic arthritis associated uveitis (JIA-uveitis) compared to the previous decade in the light of new treatment guidelines.

**Methods:** In this retrospective cohort 143 patients with JIA-uveitis were stratified into two cohorts based on the year of diagnosis of uveitis, <2010 (n=61) and ≥2010 (n=82). Development of ocular complications and visual outcomes were analyzed by univariate and multivariate methods. Treatment with systemic corticosteroids and immunomodifying medication (IMT) were documented.

**Results:** In total 109 and 133 affected eyes, respectively for cohort 1 (<2010) and cohort 2 (≥2010) were included for analysis. In the multivariate analysis with correction for paired eyes, patients in cohort 1 were at higher risk for cataract surgery ($P$ = 0.03) and secondary glaucoma ($P = 5.15 \times 10^{-3}$). Also, the number of eyes that were legally blind and visually impaired at five years follow-up was significantly higher in cohort 1 (7% vs. 2% and 8% vs. 0%, $P$ = 0.01, respectively). The number of patients that started IMT was significantly higher in cohort 2 (57% versus 98%, $P = 2.17 \times 10^{-6}$). In cohort 2, both methotrexate and anti-TNF-α therapy were prescribed earlier in the disease course (1.41 vs 0.05 years, $P = 8.31 \times 10^{-6}$ and 6.07 vs 1.84 years, $P = 5.14 \times 10^{-5}$, respectively).

**Conclusions:** The prognosis of JIA-uveitis has improved during the last decade. There is a reduction in the number of cataract surgeries and secondary glaucoma and fewer patients lose their vision parallel with earlier access to tertiary care and earlier introduction of IMT.

## INTRODUCTION

Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in children with some genetic predisposition, but still unclarified triggers.[1,2] Depending on JIA subtype, approximately one third of JIA patients develops uveitis within four years after JIA onset.[3,4] Uveitis in JIA (JIA-uveitis) is the leading cause of uveitis in children, with high risk of development of complications (e.g. cataract and glaucoma).[5-15] Consequently, by adulthood, approximately 30% of the eyes with JIA-uveitis become visually impaired which poses a significant burden on the quality of life.[4,16,17] Male gender, short interval between arthritis and uveitis diagnosis, uveitis diagnosis prior to arthritis diagnosis, young age at uveitis onset, disease severity and presence of complications at first ophthalmological visit have been reported as risk factors for visual impairment and ocular complications.[3,8-10,12,14,18-22] Therefore, the combination of established standardized uveitis screening for early detection, specialized care by a uveitis expert, and the development of novel immunomodifying treatments are expected to improve outcomes. Formerly, topical and/or systemic corticosteroids were used for rapid control of intraocular inflammation.[23-25] However, given the potentially severe side effects of both topical (cataract, glaucoma), and systemic corticosteroids (e.g. Cushing's syndrome), adding immunomodifying treatment (IMT) like methotrexate (MTX) earlier in the disease course is widely recommended nowadays so that topical and systemic corticosteroids can be tapered.[23-32] Further, the therapeutic potential of tumor necrosis factor alpha (TNF-α) inhibitors, in addition to methotrexate, is significant.[33] Treatment with biologics in JIA has been registered since 2010 and changed the clinical practices completely. Therefore, in this retrospective study we compared the clinical outcomes in children with JIA-uveitis diagnosed before 2010 to children diagnosed in 2010 and after.

## METHODS

**Patient and data collection**

The study was approved by the Medical Ethical Research Committee of the University Medical Center Utrecht in concordance with the Declaration of Helsinki. In this retrospective study, we compared the clinical outcomes of a previously reported cohort of children with JIA uveitis diagnosed <2010 to a new cohort of children diagnosed ≥2010.[18] All patients were seen by a pediatric rheumatologist who diagnosed the patients with JIA based on the criteria of the *International League of Associations for Rheumatology* or by former criteria (e.g., *European League Against Rheumatism* (EULAR).[34-36] All patients had an uveitis screening according to the guideline of American Academy of Pediatrics.[37] Uveitis was diagnosed by an ophthalmologist specialized in pediatric uveitis at the Department of Ophthalmology, University Medical Centre, Utrecht, the Netherlands, a tertiary referral center. Patients with SUN ≥ 1+ cells or more in the anterior chamber, were diagnosed with JIA-associated anterior uveitis.[38]

Clinical data were retrospectively collected from the patient files. The following information from the medical data of the patients were recorded: gender, age of onset of uveitis, age of onset of JIA, laterality, laboratory results of antinuclear antibodies (ANA), ocular complications, best corrected visual acuities (BCVA) and systemic treatment. The following ocular complications were registered: ocular hypertension, posterior synechiae, secondary glaucoma, glaucoma requiring surgery, cataract requiring surgery, cystoid macula edema (CME), papillitis and hypotony. Ocular hypertension was defined as three successive intraocular pressure measurements higher than 21 mmHg for which anti-glaucomatous treatment had been started in the absence of pathologic optic disk cupping or visual field changes.[39] Secondary glaucoma was classified as the presence of pathologic disc cupping and / or glaucomatous visual field, in combination with history of intraocular pressure higher than 21 mmHg. Pre-perimetric glaucoma was defined as glaucomatous changes on the optical coherence tomography (OCT) retinal nerve fiber layer (RNFL). Since the OCT was not available in our clinic until 2003, CME was defined as the presence of macular thickening with cyst formation observed by fundoscopy or by OCT, before and after 2003 respectively. Visual impairment was defined as BCVA between 20/50 and 20/200, and legal blindness as BCVA of 20/200 or worse or a visual field less than 10 degrees.[18]

The data regarding treatment with corticosteroid and IMT were collected. Because of missing values regarding the amount of drops and duration of treatment with topical corticosteroids, treatment with topical corticosteroids was excluded from the analysis.

**Statistical analyses**

Statistical analyses of the data were performed using R software version 3.6.1. Baseline characteristics and administration of therapy were analyzed per patient. Data are presented as medians with an interquartile range. The Pearson $X^2$ test or Fisher exact test were used for univariate analysis of categorical variables and The Mann-Whitney U test was used to compare medians. Statistical analysis of ocular complications and BCVA were performed "by eye", only including affected eyes with a five years follow-up with correction for paired eyes. Generalized estimating equations (GEE) was used for correction of paired eyes.[40] A Cox proportion hazard regression was applied for multivariate analysis including all affected eyes with correction of standard error with clustering using robust method.[41] The model was adjusted for age of onset of uveitis (numeric variable), gender and uveitis diagnosis before arthritis onset. P-values of less than 0.05 were considered statistically significant. All tests were 2- tailed.



## RESULTS

**General characteristics of the study population**

Sixty-one patients diagnosed with JIA-uveitis before 2010 were included in cohort 1. Cohort 2 included 82 patients diagnosed with JIA-uveitis in 2010 and after (**Table 1**).[18] Children in cohort 1 were younger at uveitis onset and had a longer interval between diagnosis of uveitis and initial visit at a tertiary referral center than the children in cohort 2. Other comparisons of baseline characteristics can be seen in **Table 1**.

**Table 1.** General characteristics of the study population according to cohort.

| | Cohort 1 | Cohort 2 | |
|---|---|---|---|
| Timing of uveitis | <2010 | ≥2010 | *P-value* |
| Total number of patients | 61 | 82 | |
| Total number of eyes | 109 | 133 | |
| Male, n (%) | 15 (25) | 19 (23) | 1.00 |
| Bilateral, n (%) | 48 (78) | 51 (62) | 0.05 |
| ANA seropositive, n (%) | 54 (89) | 64 (78) | 0.26 |
| Age of onset JIA, median in years (IQR) | 3.3 (2.0-5.0) | 3.0 (3.3-7.4) | 0.82 |
| Age of onset uveitis, median in years (IQR) | 4.2 (3.1-6.2) | 5.2 (3.8-7.6) | 0.03 |
| Uveitis diagnosis before JIA onset, n (%) | 15 (25) | 9 (11) | 0.05 |
| Time between diagnosis of arthritis and uveitis, median in years (IQR) | 1.3 (0.3-1.0) | 1.0 (0.4-2.0) | 0.27 |
| Time between diagnosis of uveitis and initial visit at the tertiary center, median in years (IQR) | 2.2 (0.7-5.5) | 0.9 (0.1-2.2) | $8.08 \times 10^{-4}$ |
| Follow-up time, median in years (IQR) | 10 (9.1-10) | 5.1 (2.6-6.9) | $8.90 \times 10^{-14}$ |

JIA, juvenile idiopathic arthritis; ANA, antinuclear antibodies; IQR, interquartile range.

## Complications

### Cohort 1 and cohort 2

Cumulative incidences of ocular complications according to the two different cohorts are presented in **Table 2**. After adjusting for paired eyes, we found that in cohort 1, cataract surgeries were more common than in cohort 2 at five years of follow up (40% vs 20%, $P = 0.02$, GEE). No other differences were found after adjusting for paired eyes. Of the eyes that needed glaucoma surgery at five years of follow-up, the indications were ocular hypertension (40% versus 50%), pre-perimetric glaucoma (32% versus 30%) and secondary glaucoma (28% versus 20%, $P = 0.90$).

### Uveitis diagnosis before arthritis onset

After adjusting for paired eyes, the group with uveitis diagnosis before arthritis onset developed more frequently posterior synechiae (29% vs 74%, $P = 6.00 \times 10^{-6}$), CME (10% vs 39%, $P = 1.94 \times 10^{-4}$) and needed to undergo cataract surgery more frequently (24% vs 59%, $P = 2.64 \times 10^{-3}$) at five years of follow up.

### Risk factors for ocular complications

Cox proportion hazard regression analysis with correction for age, gender and uveitis diagnosis before arthritis onset, revealed that patients in cohort 1 had an increased risk for cataract surgery (HR = 1.84, 95% CI: 1.06-3.20, $P = 0.03$) (**Figure 1A**). Furthermore,

male gender and uveitis diagnosis before arthritis onset also appeared to be an independent risk factor for cataract surgery (HR = 1.68, 95% CI: 1.00-2.80, $P = 4.79 \times 10^{-2}$ and HR = 2.25, 95% CI: 1.33-3.82, $P = 2.43 \times 10^{-3}$, respectively). Cohort 1, was the only independent risk factor for secondary glaucoma (HR = 2.81, 95% CI: 1.36-5.79, $P = 5.15 \times 10^{-3}$) (**Figure 1B**). Independent risk factors for posterior synechiae were uveitis diagnosis before arthritis and younger age of uveitis onset (HR = 3.11, 95% CI: 1.95-4.98, $P = 2.13 \times 10^{-6}$ and HR = 0.90, 95% CI: 0.83-0.99, $P = 0.03$, respectively).

**Table 2.** Cumulative incidences of ocular complications of patients with juvenile idiopathic arthritis associated uveitis at diagnosis and at five years of follow-up according to cohort.

| | Cohort 1 | Cohort 2 | |
|---|---|---|---|
| Timing of uveitis | <2010 | ≥2010 | *P-value* [a] |
| At diagnosis | | | |
| Number of eyes | 109 | 133 | |
| Posterior synechiae, n (%) | 24 (22) | 24 (18) | 0.44 |
| Cataract surgery, n (%) | 0 (0) | 0 (0) | NA |
| Ocular hypertension, n (%) | 6 (6) | 18 (14) | 0.04 |
| Secondary glaucoma, n (%) | 1 (1) | 0 (0) | 0.45 |
| Glaucoma surgery, n (%) | 0 (0) | 1 (1) | 1.00 |
| CME, n (%) | 2 (2) | 6 (5) | 0.30 |
| Papillitis, n (%) | 5 (5) | 8 (6) | 0.62 |
| Hypotony, n (%) | 0 (0) | 0 (0) | NA |
| Follow-up at five years | | | |
| Number of eyes | 105 | 76 | |
| Posterior synechiae, n (%) | 47 (45) | 22 (29) | 0.03 |
| Cataract surgery, n (%) | 42 (40) | 15 (20) | $3.79 \times 10^{-3}$ |
| Secondary glaucoma, n (%) | 22 (21) | 8 (10) | 0.06 |
| Glaucoma surgery, n (%) | 25 (24) | 10 (13) | 0.07 |
| CME, n (%) | 20 (19) | 9 (12) | 0.19 |
| Papillitis, n (%) | 16 (15) | 15 (20) | 0.43 |
| Hypotony, n (%) | 3 (4) | 0 (0) | 1.00 |

CME, cystoid macular edema; NA, not applicable. Analysis performed by "eye".
[a] The Pearson $X^2$ test or Fisher exact test.

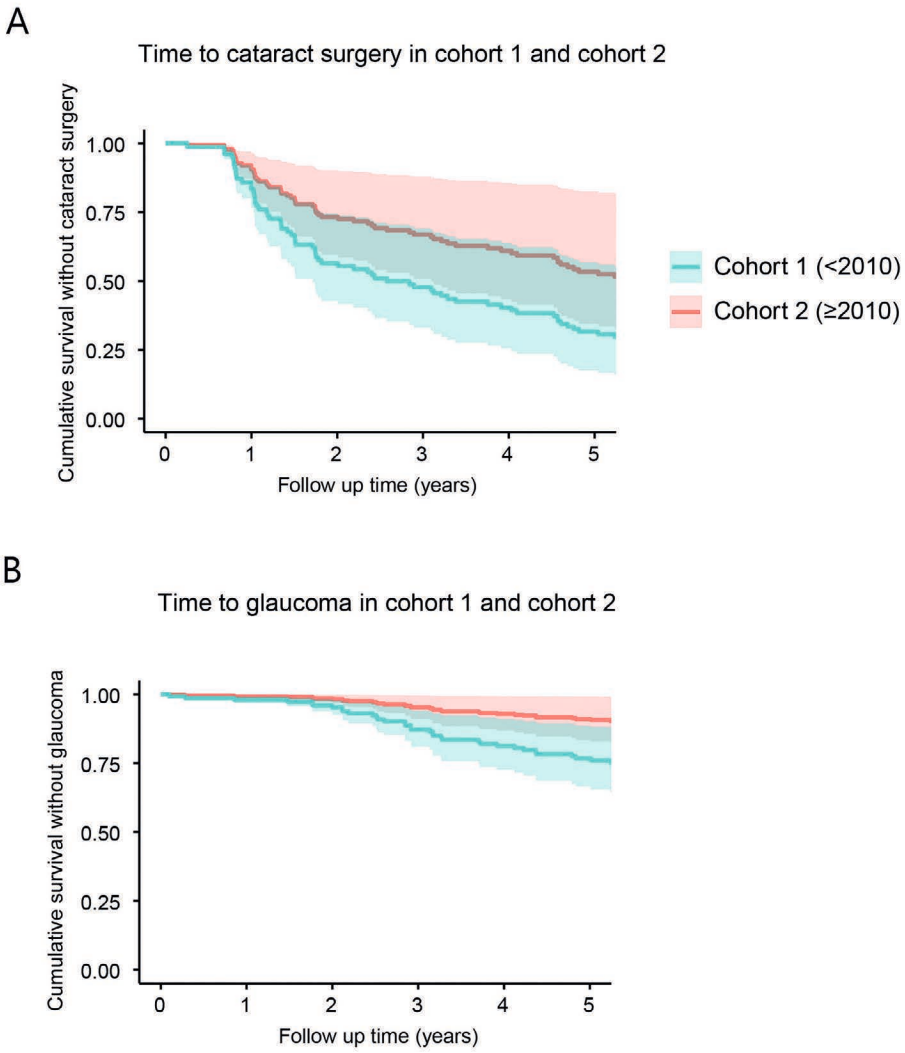


**Figure 1.** Time to the different complications.

**A)** Time to cataract surgery. HR = 1.84, 95% CI: 1.06-3.20, $P$ = 0.03. **B)** Time to secondary glaucoma. HR = 2.81, 95% CI: 1.36-5.79, $P = 5.15 \times 10^{-3}$. Analysis is adjusted for age of uveitis, uveitis diagnosis before arthritis onset, gender and paired eyes. HR, hazard ratio; CI, confidence interval.

Next, to identify the potential effect of early start with IMT we compared the interval between diagnosis of uveitis and treatment with methotrexate and anti-TNF-α between patients with and without ocular complications in the two different cohorts (**Table 3**). In cohort 1, patients with secondary glaucoma and children with cataract requiring surgery had a longer interval between diagnosis of uveitis and start with methotrexate and start with anti-TNF-α therapy compared to patients without these complications, although this was not significant (**Table 3**). These differences were not observed in cohort 2. Patients from cohort 2 with cataract requiring surgery had a significant shorter time between diagnosis of uveitis and initial visit at the tertiary center (**Table 3**).



**Visual outcomes**

Median BCVA's and categorized BCVA's at standard points of follow-up, analyzed according to cohort, are presented in **Table 4**. After adjusting for paired eyes, the total number of legally blind and visually impaired eyes were higher in cohort 1 (7% versus 2% and 8% vs. 0%, $P = 0.02$, GEE). No other differences were identified (**Table 4**).

**Table 3**. Time between diagnosis of uveitis and start with immunosuppressive therapy and time between diagnosis of uveitis and initial tertiary center visit in patients with and without complications in the two different cohorts.

| | Cohort 1 | | | Cohort 2 | | |
|---|---|---|---|---|---|---|
| Cataract surgery | Yes | No | *P-value* | Yes | No | *P-value* |
| Number of patients, n (%) | 36 (59) | 25 (41) | | 17 (21) | 65 (79) | |
| Time between diagnosis of uveitis and start methotrexate, median in years (IQR) | 2.5 (0.5-5.8) | 0.4 (0.0-3.3) | 0.10 | 0.0 (0.0-0.1) | 0.1 (0.0-0.7) | 0.22 |
| Time between diagnosis of uveitis and start anti-TNFα therapy, median in years (IQR) | 7.0 (3.7-9.5) | 5.1 (3.2-7.0) | 0.45 | 1.8 (0.7-3.7) | 1.9 (0.7-4.5) | 0.85 |
| Time between diagnosis of uveitis and initial visit at tertiary center, median in years (IQR) | 1.3 (0.7-5.5) | 3.0 (2.2-4.8) | 0.57 | 0.1 (0.0-0.9) | 0.9 (0.2-2.4) | 0.04 |
| Secondary glaucoma | | | | | | |
| Number of patients, n (%) | 28 (46) | 33 (54) | | 9 (9) | 73 (91) | |
| Time between diagnosis of uveitis and start methotrexate, median in years (IQR) | 2.5 (0.5-5.7) | 0.6 (0.0-3.9) | 0.16 | 0.0 (0.0-0.0) | 0.08 (0.0-0.6) | 0.02 |
| Time between diagnosis of uveitis and start anti-TNF-α therapy, median in years (IQR) | 8.0 (5.2-9.5) | 3.9 (2.8-5.7) | 0.19 | 1.6 (0.6-2.1) | 2.1 (0.7-4.5) | 0.54 |
| Time between diagnosis of uveitis and initial visit at tertiary center, median in years (IQR) | 1.7 (0.9-3.3) | 4.4 (0.7-8.3) | 0.29 | 0.5 (0.1-1.3) | 0.9 (0.1-7.2) | 0.80 |

anti-TNF-α, anti-tumor necrosis factor alpha; IQR, interquartile range.

**Table 4.** Median best-corrected visual acuity and categorized visual outcomes in eyes affected by juvenile idiopathic arthritis associated uveitis, according to cohort.

| | Cohort 1 | Cohort 2 | |
|---|---|---|---|
| Timing of uveitis | >2010 | ≥2010 | *P-value* [a] |
| At diagnosis | | | |
| Number of eyes | 109 | 133 | |
| BCVA, median (range) | 1.0 (0.8-1.0) | 1.00 (0.8-1.0) | 0.22 |
| ≤20/200, n (%) | 3 (5) | 3 (5) | 0.98 |
| 20/100–20/50, n (%) | 4 (7) | 5 (8) | |
| ≥20/40, n (%) | 50 (88) | 58 (88) | |
| Follow-up at five years | | | |
| Number of eyes | 105 | 76 | |
| BCVA, median (range) | 1.0 (0.6-1.0) | 1.20 (1.0-1.2) | $1.48 \times 10^{-9}$ |
| ≤20/200, n (%) | 6 (7) | 1 (2) | 0.01 |
| 20/100–20/50, n (%) | 8 (8) | 0 (0) | |
| ≥20/40, n (%) | 78 (85) | 63 (98) | |

BCVA, best-corrected visual acuity. Analysis performed "by eye".
[a] The Pearson $X^2$ test or Fisher exact test

3

### Treatment

The differences in treatment with systemic corticosteroids and IMT at five years of follow-up are presented in **Table 5**. There was no difference in the number of patients that were treated with systemic corticosteroids, however in cohort 2, systemic corticosteroids were predominately prescribed between 2011 and 2014. All patients requiring systemic treatment were initiated with methotrexate. In cohort 2, both methotrexate and anti-TNF-α therapy were prescribed earlier in the disease course compared to cohort 1 (1.41 [0.07-4.72] years vs 0.05 [0.00-0.37] years, $P = 8.31 \times 10^{-6}$ and 6.07 [3.40-9.23] years vs 1.84 [0.66-4.28] years, $P = 5.14 \times 10^{-5}$, respectively (**Figure 2**).

**Table 5.** Treatment with systemic corticosteroids and immunomodifying treatment at five years of follow-up according to cohort.

| | Cohort 1 | Cohort 2 | |
|---|---|---|---|
| Timing of uveitis | <2010 | ≥2010 | *P-value* |
| Follow-up at five years | | | |
| Number of patients, n (%) | 58 (95) | 45 (55) | |
| Systemic corticosteroids, n (%) [a] | 13 (23) | 12 (27) | 0.65 |
| Methotrexate, n (%) | 33 (57) | 44 (98) | $2.17 \times 10^{-6}$ |
| Anti-TNF-α therapy | 6 (10) | 22 (49) | $1.30 \times 10^{-5}$ |
| Adalimumab, n (%) | 6 (100) | 20 (91) | |
| Infliximab, n (%) | 0 (0) | 2 (9) | |

anti-TNF-α = anti-tumor necrosis factor alpha.
[a] Perioperative use of systemic corticosteroids was not included.

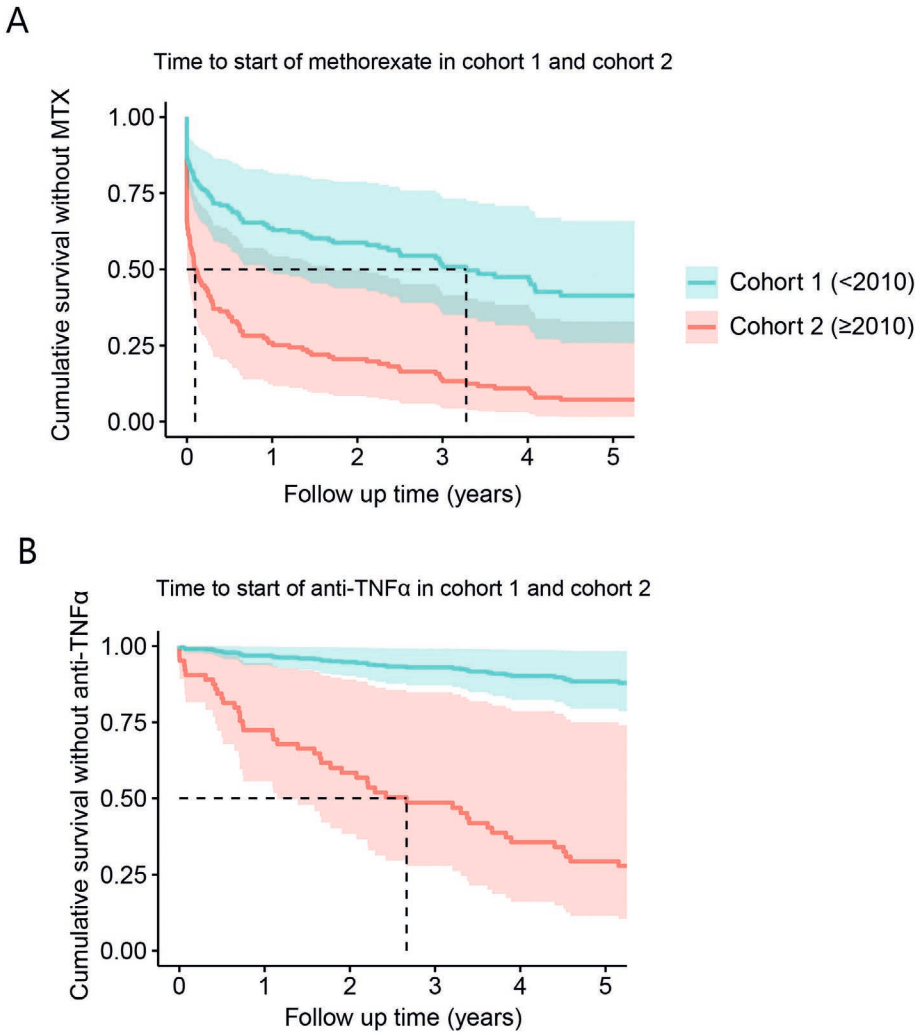


**Figure 2.** Time to immunomodifying treatment.

**A)** Time to treatment with methotrexate. HR = 0.34, 95% CI: 0.22-0.52, $P = 1.04 \times 10^{-6}$. **B)** Time to treatment with anti-TNF-α therapy. HR= 0.10, 95% CI: 0.05-0.20, $P = 1.98 \times 10^{-10}$. Analysis is adjusted for age of uveitis, uveitis diagnosis before arthritis onset, and gender. The dotted line represents the median survival. HR, hazard ratio; CI, confidence interval.

## DISCUSSION

Our retrospective cohort study shows a reduction in the rates of cataract surgeries, secondary glaucoma development, visual impairment and blindness among patients with JIA-uveitis diagnosed ≥2010 compared with patients diagnosed <2010 along with more intensive therapy and earlier access to a tertiary center. Since most of the prognostic studies in patients with JIA-uveitis are from the previous decade, before the new treatment guidelines, a new prognostic study is of great value both for ophthalmologists and patients.[25]

In our study, the rate of visual impairment reduced substantially from 8% to 0%. A reduction in the number of eyes with visual impairment was also reported in an earlier study by Tappeiner.[42] According to studies from previous decades, cataract occurs in 15-46% and glaucoma in 7-27% of affected eyes of patients with JIA uveitis, depending on follow up.[5-7,10,14,43-46] A recent study of Tappeiner revealed a significant decrease in the prevalence of cataract (from 28% to 16%) and secondary glaucoma (from 10% to 5%) between 2009 and 2013.[42] In our study, the rate of cataract surgery (20% in cohort 2) and secondary glaucoma (10% in cohort 2) were higher than the recent studies of Tappeiner and Heiligenhaus.[9,42] The difference in the number of complications between studies can be caused by a difference in corticosteroid treatment, follow-up or definition of a complication which are not always reported in the studies.[5-7,9,10,14,43-46] This emphasizes the unmet need for a study that describes the prognostic changes in children with JIA-uveitis using the recommended standardized outcomes[47-48] Male gender, short interval between arthritis and uveitis diagnosis, uveitis diagnosis prior to arthritis diagnosis, young age at uveitis onset and disease severity are associated with a poor prognosis.[3,8-10,12,14,18-22] In our study, patients in cohort 1 were significantly younger of age at uveitis diagnosis. The difference in age at uveitis diagnosis might be explained by the fact that nowadays MTX is well-established as first-line treatment in the management of JIA which might delay or even prevent the occurrence of uveitis.[33]

General opinion is that early treatment with immunomodifying agents can reduce the rate of complications and this is in line with this study.[23-25,45] However, due to the retrospective design of our study, a causal role of immunomodifying therapy to prevent ocular complications cannot be proven. Early treatment with immunomodifying agents is also recommended so that topical and systemic corticosteroids can be tapered to prevent long-term treatment with corticosteroids.

In our study, there was no difference in the number of patients that were treated with systemic corticosteroids between the cohorts. Besides earlier treatment with immunomodifying agents, we hypothesize that the clinical improvement is also influenced by better access to multidisciplinary specialized care between ophthalmologist and pediatric rheumatologists.

Unfortunately, due to the retrospective design of the study we were unable to take into account the use of topical corticosteroids. The number of corticosteroid drops and duration of treatment is related to cataract and glaucoma development as has been shown in several studies.[26,27]

In conclusion, our study reports a significant decrease in the number of cataract surgeries, secondary glaucoma development and lower rates of visual impairment parallel with earlier access to a tertiary referral center and more intensive IMT. These data on clinical outcome and visual prognosis are relevant for ophthalmologists who need to council the child and their parents for the need of intensive IMT for uveitis. Additionally, our study will contribute in creating awareness among ophthalmologists about the increased risks of complications in patients with JIA-uveitis. Further studies are required to verify the causal association of early IMT on the course of uveitis in JIA.

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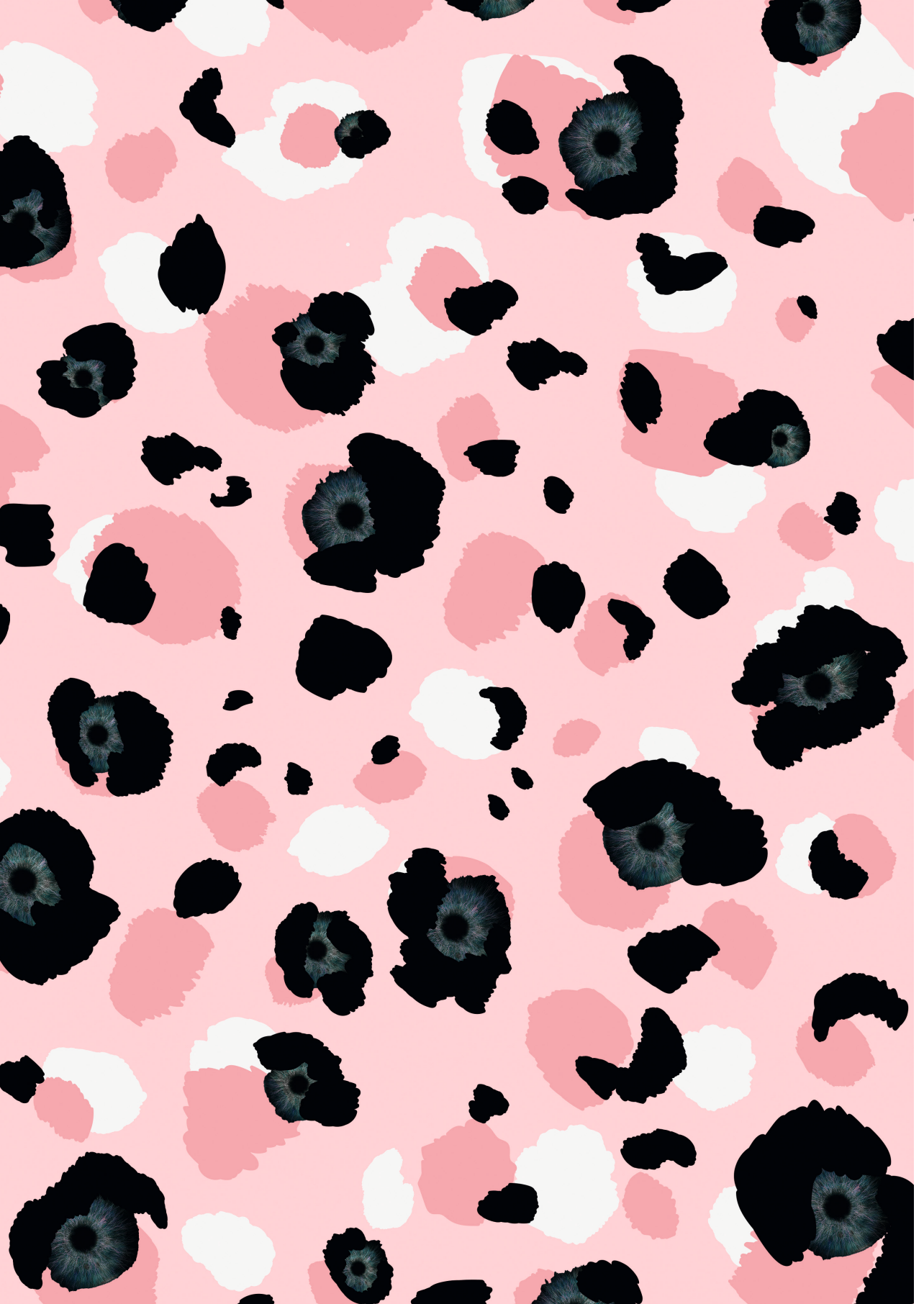

# CHAPTER 4

## Tocilizumab as effective treatment in patients with refractory intermediate uveitis

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## ABSTRACT

**Purpose:** To describe the results of tocilizumab treatment in children with refractory non-anterior uveitis.

**Methods:** A case series of seven children with refractory non-anterior uveitis (onset ≤16 years) with leakage on fluorescein angiogram (FA) were treated with tocilizumab intravenously every four weeks (eight mg/kg). Minimum follow-up was six months. Reported outcomes are changes in BCVA, central macular thickness (CMT) on OCT image, FA scores, dose of systemic steroids, complications and side effects.

**Results:** In all patients there was an improvement of macular edema and capillary leakage on FA. The median FA score decreased from 14 (10-18) at baseline to 8 (2-9) after six months of treatment ($P$ = 0.018). The CMT decreased from 321 (314-384) to 295 (255-312) ($P$ = 0.043). BCVA improved in five eyes and worsened in one eye due to cataract. No systemic or ocular complications were reported.

**Conclusion:** Tocilizumab is an effective therapeutic option for reducing disease activity in children with refractory non-anterior uveitis.

## INTRODUCTION

Pediatric uveitis is a very challenging condition to treat. It leads to irreversible visual impairment in up to one third of the affected children.[1,2] Underlying etiology as well as treatment response varies widely in these patients. Corticosteroids have been traditionally used as the first-line treatment, but given their severe adverse effects (i.e. growth retardation and Cushing syndrome) choosing for immunomodulating treatment (IMT) like mycophenolate mofetil (MMF) or methotrexate (MTX) earlier in the disease course is widely accepted nowadays. However, also IMT is frequently not sufficient to control uveitis; more than half of the patients eventually need to start additionally with biologicals.[3] The therapeutic potential of adalimumab (TNF-α inhibitor) in adults with noninfectious uveitis and children with juvenile idiopathic arthritis (JIA) associated uveitis is well known.[4-8] However, caution should be kept in mind for TNF-α inhibitors as a treatment for intermediate uveitis (IU) due to a potentially increased risk of demyelinating disease of the central nerve system.[6] So there is an unmet need for additional effective treatment options in children with refractory IU and panuveitis dependent on chronic treatment with corticosteroids.



Tocilizumab (TCZ) is a recombinant humanized antibody directed against the IL-6 receptor. While the effectiveness of TCZ was established in the treatment of cystoid macula edema (CME) and JIA associated uveitis, there is still little known about its effect in pediatric non-infectious IU and panuveitis.[9-11] In this retrospective cohort, we describe the results of TCZ treatment in children with refractory IU and panuveitis.

## METHODS

This is a retrospective cohort of children with visual threatening idiopathic IU and panuveitis (onset before 16 years of age) with severe leakage on the fluorescein angiography (FA) and refractory to treatment with a combination of systemic corticosteroids, one or more IMT and TNF-α inhibitors (adalimumab) or with relative contraindications for TNF-α inhibitors see **Table 1**. All patients were treated with TCZ between 2016 and 2018 at the department of ophthalmology of the University Medical Centre of Utrecht (n=6) and St. RadboudUMC Nijmegen (n=1), tertiary reference centers in the Netherlands. Parents and patients were aware of the use of an off-label drug and gave their consent. Uveitis was diagnosed by an ophthalmologist specialized in pediatric uveitis. Diagnosis of uveitis was made according to the criteria of the International Uveitis Study Group.[12] After screening of all patients by a pediatric rheumatologist, none of the patients had a related systemic auto-inflammatory or autoimmune disease.

All patients received eight mg/kg TCZ intravenously (weight of all patients was above 30 kg) given every four weeks (second infusion after two weeks) in addition to IMT and systemic corticosteroids (**Table 1**). The minimum follow-up was six months.

Reported outcomes are changes in disease activity scored by anterior chamber cells according to Standardization of Uveitis Nomenclature (SUN) classification[12] and was graded by the ophthalmologist specialized in pediatric uveitis, central macular thickness (CMT) on optical coherence tomography (OCT) image and FA scores according to Angiography Scoring for Uveitis Working Group (ASUWOG).[13] Other outcomes are best corrected visual acuity (BCVA), dose of systemic corticosteroids and ocular and systemic complications and/or side effects. Data are retrospectively collected from the electronic patient file at baseline, six and every following six months after start of treatment with TCZ. The data are presented in continuous or ordinal variables (BCVA, CMT on OCT image, FA score and dose of systemic corticosteroids). The eye with the highest FA score at baseline (worst eye) was used for analysis. One patient had poor vision in one eye (left) due to previous retinal detachment. Only the other eye (right eye) was used for the visual acuity analysis. Wilcoxon signed rank test was applied for continuous and McNemar's Chi-square test for categorical variables. P-values below 0.05 were considered as statistically significant.

**Table 1.** Demographic characteristics of pediatric patients with intermediate and panuveitis treated with tocilizumab.

| Case | Sex | Diagnosis | Age at diagnosis | Prior medication | interval uveitis-TCZ (years) | Total duration of follow up after initiation of TCZ (months) | Medication change | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Baseline | 6 months | 12 months | Last follow up visit (6-24 months) |
| 1 | M | IU | 5 | Cortico; MMF;MTX; Cyclo;ADA | 11.72 | 24 | Cyclo 120mg; MMF 1500mg; Cortico 5mg EOD TCZ 8mg/kg | Cortico 5mg EOD | Cortico 5mg EOD | No cortico (24) |
| 2 | F | IU | 4 | Cortico; MMF; MTX; MA; ADA | 7.87 | 24 | Cortico10mg; MA 720mg; TCZ 8mg/kg | Cortico 10mg | Cortico 5mg | No cortico (24) |
| 3 | M | IU | 3 | Cortico; MMF;MTX; ADA | 10.49 | 24 | Cortico 5mg; MMF1200mg; TCZ 8mg/kg | Cortico 5mg EOD | Cortico 5mg EOD | Cortico 5mg EOD (24) |
| 4 | M | IU | 9 | Cortico; MMF | 16.95 | 12 | MMF 2000mg; TCZ 8mg/kg | No cortico | No cortico | No cortico (12) |
| 5 | M | IU | 11 | Cortico; MMF; MTX; MA; ADA | 5.49 | 24 | Cortico 10mg TCZ 8mg/kg | No cortico | No cortico | No cortico (24) |
| 6 | F | Pan | 7 | Cortico; MMF; MTX; ADA | 3.36 | 12 | Cortico 30mg; MMF 1500mg; TCZ 8mg/kg | Cortico 12.5mg | Cortico 12.5mg | Cortico 12.5mg (12) |
| 7 | F | Pan | 6 | Cortico; MTX; MMF | 9.98 | 6 | MMF; TCZ 8mg/kg | No cortico | No cortico | No cortico (6) |

M, male; F, female; IU, intermediate uveitis; Pan, panuveitis; cortico, corticosteroids; MMF, Mycophenolate mofetil; MA, Mycophenolic acid; MTX, methotrexate; Cyclo, cyclosporin; ADA, Adalimumab; TCZ, tocilizumab; mg, milligram; EOD, every other day.

## RESULTS

In the period of 2016-2018, seven patients with bilateral refractory idiopathic IU (n=5) and panuveitis (n=2) were treated with TCZ after previous failure of corticosteroid and other traditional and anti-TNF-α immunosuppressive therapy. Two patients had a relative contraindication for TNF-α inhibitors, because of suspicion of neuritis and demyelination on MRI brain**.** Demographic and baseline characteristics of patients are reported in **Table 1**. The age at diagnosis ranged from three to 11 years. Complications of long-term corticosteroids use i.e. growth retardation and Cushing were reported in all patients. The previous immunosuppressive treatment consisted of methotrexate (n=6), mycophenolate (n=7), mycophenolic acid (n=2), cyclosporine (n=1) and adalimumab (n=5). The interval between onset of uveitis and starting treatment with TCZ ranged between three and 11 years with median of 7.9 (interquartile range [IQR] 6.4-10.0) years (**Table 1**). In all patients there was a striking reduction in overall FA score from 14 (10-18) at baseline to 8 (2-9) after six months of treatment ($P$ = 0.018) and 5 (1.50-6.75) after 12 months ($P$ = 0.028). This was mostly due to reduction in macula edema and capillary leakage. There was a significant decrease of the CMT measurement on OCT image (**Figures 1, 2** and **Table 2**). At baseline, three patients had intraretinal cysts shown on FA. In one of these patients macular cysts were identifiable on the OCT image. The retinal alterations had disappeared in all three patients after six months of treatment. Retinal neovascularization at baseline was still present at the 12 month follow-up visit in one eye of one patient.

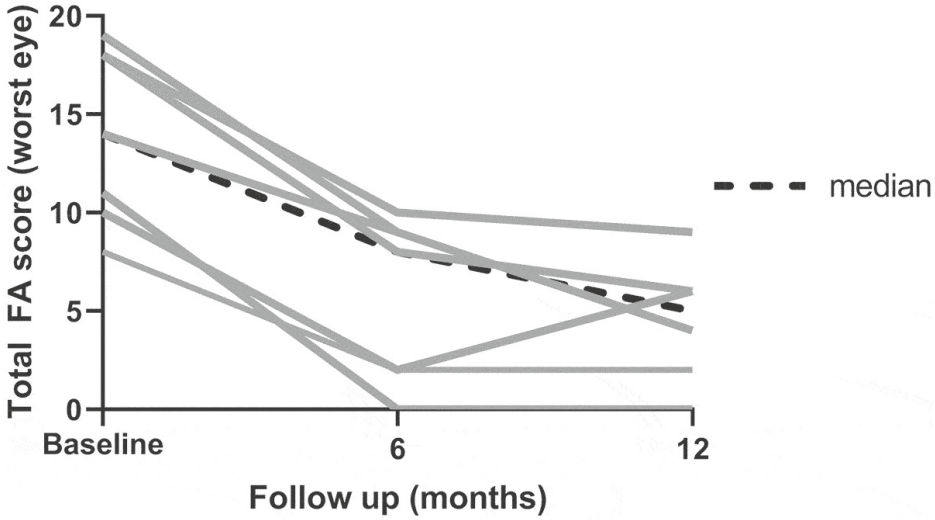


**Figure 1**. Total median fluorescein angiography score per case (worst eye) and the median total fluorescein angiography at baseline, six months and 12 months follow-up.



**Table 2.** Fluorescein angiography score, central macular thickness measurement on OCT image and best-corrected distance visual acuity of the eye with the highest FA score at baseline, six months and 12 months of follow up.

| | Baseline (n=7) | 6 months (n=7) | *P value*[b] | 6 months (n=6) | *P value*[b] |
|---|---|---|---|---|---|
| FA CME[a] | 3 (1-4) | 0 (0-1) | 0.017 | 0.5 (0-1.25) | 0.027 |
| FA capillary leakage[a] | 7.0 (6-10) | 4.0 (0-7) | 0.017 | 2.5 (0.75-4.25) | 0.028 |
| FA vasculitis[a] | 2 (1-3) | 1 (0-2) | 0.034 | 0 (0-0.5) | 0.056 |
| FA optic disc leakage[a] | 2 (0-3) | 1 (0-2) | 0.034 | 1 (0-2) | 0.083 |
| FA total score[a] | 14 (10-18) | 8 (2-9) | 0.018 | 5 (1.5-6.75) | 0.028 |
| BCVA in LogMAR | 0.15 (0.00-0.22) | 0.05 (-0.08-0.22) | 0.786 | 0.02 (0.00-0.30) | 0.500 |
| CMT in µm | 321 (314-384) | 295 (255-312)[c] | 0.043 | | |

Data given as median (interquartile range). The eye with the highest FA score at baseline was used for analysis. FA, fluorescein angiography; CME, cystoid macula edema; BCVA, best-corrected distance visual acuity; LogMAR, logarithm of minimal angle of resolution; CMT, central macular thickness; µm, microns.
[a] FA scores as described by The Angiography Scoring for Uveitis Working Group.[13]
[b] *P-values* computed with Wilcoxon signed rank test.
[c] Measurement after nine months of treatment.

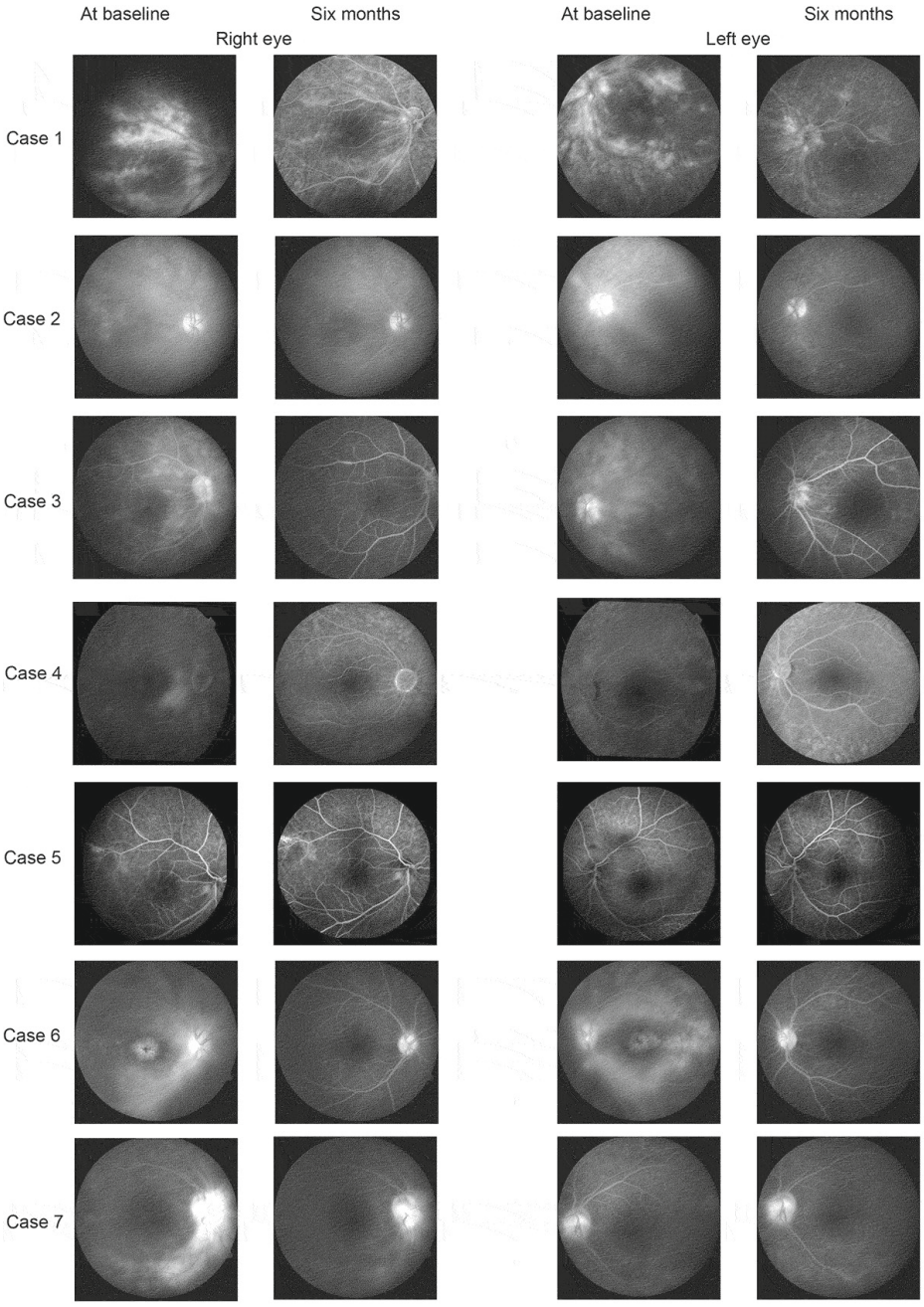


**Figure 2.** The two left columns show the fluorescein angiography of the right eye per case at baseline and after 6 months of treatment. The two right columns show the fluorescein angiography of the left eye per case at baseline and after 6 months of treatment.

Improvement of BCVA was observed in five (out of 13) eyes (two patients bilaterally), stabilization in seven eyes and worsened in one eye due to cataract (0.15 to 0.22 LogMAR) in a follow-up period of 6-12 months. The median BCVA, however, did not significantly change (**Table 2**). Cataract was present at baseline in five eyes of three patients with no change during follow up.

In three out of five patients the dose of systemic corticosteroids could be ceased and reduced in two patients in the last follow-up visit **(Table 1**). No systemic or ocular complications were reported during the follow-up period.

## DISCUSSION

This small case series shows that treatment with TCZ is successful in children with refractory IU and panuveitis who failed on TNF-α inhibitors or had a relative contra-indication for TNF- α inhibitors. In all patients, TCZ seems very effective to improve macular edema and capillary leakage on FA.



Approximately 94% of children with idiopathic uveitis need systemic steroids during the course of disease and more than 50% require IMT and biologicals.[3] TNF-α inhibitors showed to be effective in different forms of (childhood) uveitis.[4-8] They are, however, being given with caution in case of traditional IMT failure because of reports on demyelination in patients with non-anterior uveitis. Therefore, in all patients with non-anterior uveitis an MRI scan of the brain is recommended before starting TNF-α inhibitors. Two out of seven patients had white matter abnormalities on MRI pre-existent to treatment. This was classified as a relative contraindication for TNF-α inhibitors. Five out of seven patients were treated with adalimumab with unsatisfactory results.

TCZ is emergently regarded and used as a valuable treatment option for JIA associated uveitis.[9-11] Additionally, TCZ in adults with non-anterior uveitis and refractory uveitis related cystoid macula edema is well established.[14-21] The STOP-Uveitis Study demonstrated that monthly intravenous TCZ infusions significantly improved the visual, anatomic, and inflammatory outcomes in patients with non-anterior uveitis with limited systemic and ocular adverse effects.[14] Comparable results were seen with sarilumab subcutaneous, another anti-IL-6 receptor, in adult uveitis.[22]

Meanwhile data on the effectiveness and tolerance of TCZ in pediatric non-anterior uveitis are still lacking.

In our study, there was a striking improvement of disease activity and reduction of macular leakage on FA after six months of treatment with TCZ intravenously. This emphasizes the important role of regularly performed FA in evaluating treatment results.

A significant decrease in CMT measurement on OCT image was seen in our study. However, other studies which reported this effect, show a more substantial reduction.[14, 16-17, 21,22] This might be explained by the absence of macular cysts in most of our patients. None of the patients had any tractional component. At present, it is unknown whether TCZ subcutaneously will have the same effect on uveitis activity but favorable results were reported with sarilumab subcutaneously.[22] Like in our study, corticosteroid-sparing effect of TCZ has been reported in many other studies.[14,15,17,18,20-22] This is an important aspect since in our cohort, all patients had unwanted steroid-related complications i.e. growth retardation, Cushing syndrome and osteoporosis at this young age.

Although, favorable effect on vision after treatment with anti-IL-6 receptor has been reported, in our study, improvement in BCVA was observed in the minority of the eyes (five of 13) within the follow-up. [14,16,17,21,22] This can be explained by study design (children versus adults), the number of patients with CME and cataract development.

In the literature reported complications of TCZ are low absolute neutrophil counts, leukopenia, and thrombocytopenia.[15,18] None of these were observed in our study. Conversely, in trials in patients with systemic juvenile idiopathic arthritis, 66-88% of patients had at least one adverse event.[23-24] This is probably related to systemic inflammatory condition and not comparable with our population.

The primary limitation of our study is its small sample size. However, uveitis is a rare entity and moreover, the clinical value for new therapies with less side effects in this challenging patient population is of great importance. Another limitation of this study is the relatively short follow-up period of six months to 24 months. Despite the retrospective study design, treatment and follow up were according protocol.

In conclusion, the results of our study show rapid and significant improvement in disease activity in children with intermediate and panuveitis treated with intravenous TCZ which was well tolerated. Future larger studies are needed to confirm the effectiveness and safety of intravenous TCZ in children with noninfectious uveitis. Our observation is therefore likely to be valuable for ophthalmologists and pediatric immunologists who treat children with uveitis.

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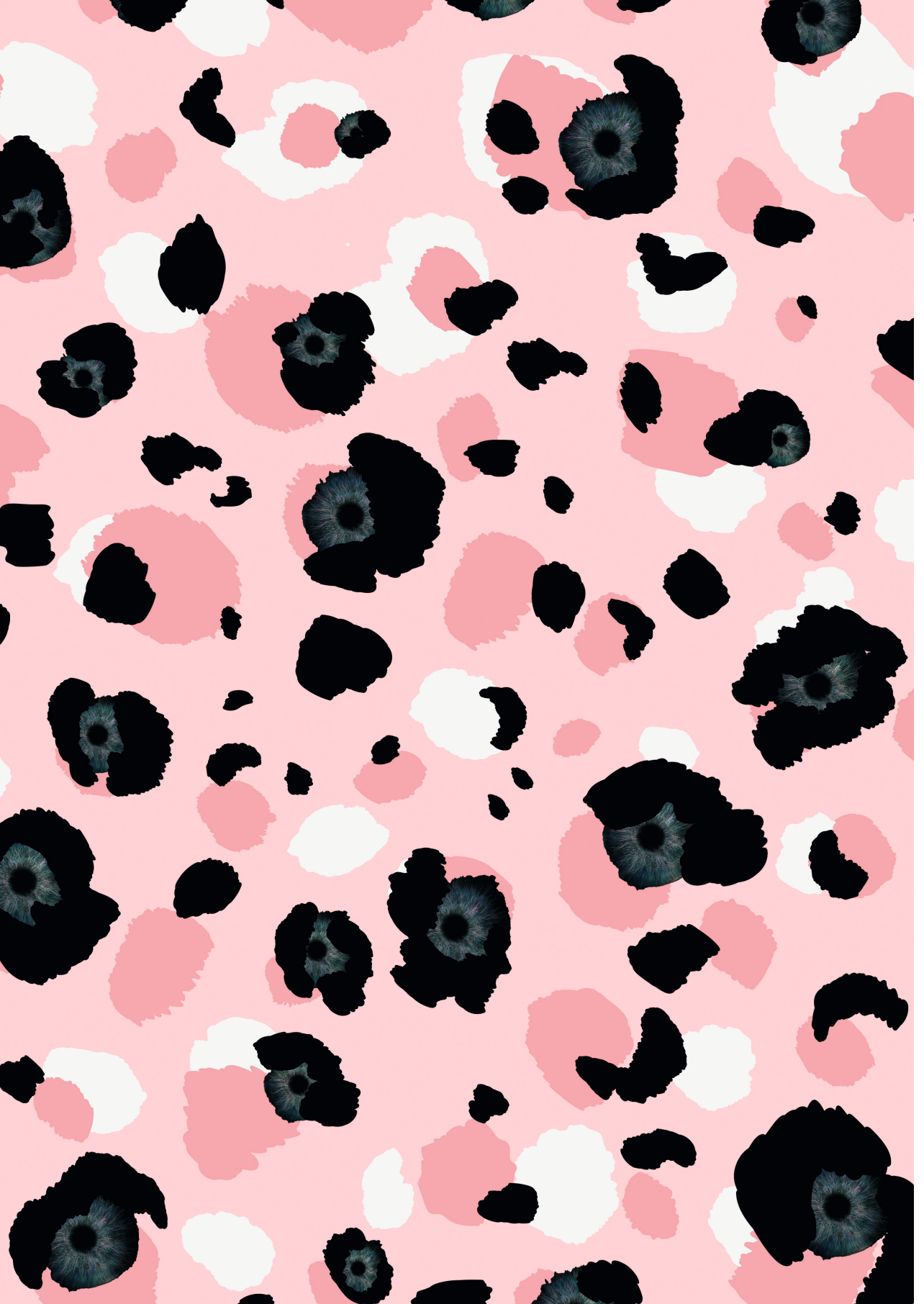

# CHAPTER 5

## A serum protein signature stratifies treatment response to csDMARDS in pediatric non-infectious uveitis

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## ABSTRACT

**Purpose:** To identify a serum biomarker signature that can help predict response to conventional synthetic disease modifying antirheumatic drugs (csDMARD) therapy in pediatric non-infectious uveitis.

**Methods:** In this case-control cohort study, we performed a 368-plex proteomic analysis of serum samples of 72 treatment-free patients with active uveitis (new onset or relapse) and 15 healthy controls. Among these, 37 patients were sampled at diagnosis before commencing csDMARD therapy. After six months, csDMARD response was evaluated and cases were categorized as "responder" or "non-responder". Patients were considered "non-responders" if remission was not achieved under csDMARD therapy. Serum protein profiles were used to train random forest models to predict csDMARD failure and compared to a model based on eight clinical parameters at diagnosis (e.g., maximum cell grade).

**Results:** In total, 19/37 (51%) cases were categorized as csDMARD non-responders. We identified a 10-protein signature that could predict csDMARD failure with an overall accuracy of 84%, which was higher compared to a model based on eight clinical parameters (73% accuracy). Adjusting for age, sex, anatomical location of uveitis and cell grade, cases stratified by the 10-protein signature at diagnosis showed a large difference in risk for csDMARD failure (hazard ratio = 12.8 [95% confidence interval 2.5-64.6], $P = 0.002$).

**Conclusion:** Machine learning models based on the serum proteome can stratify pediatric uveitis patients at high risk for csDMARD failure.

## INTRODUCTION

Non-infectious pediatric uveitis accounts for 5-10% of all uveitis patients.[1] Despite this relatively small proportion, it has often a severe disease course and a disproportionately large disease burden due to the young age of onset.[2,3] Direct evidence is lacking, but an immune-mediated etiology is strongly suspected in non-infectious pediatric uveitis.[4-9] Over half of cases suffer from chronic disease and consequently require long periods of treatment with immunosuppressive agents, most often conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) such as methotrexate or mycophenolate mofetil.[10-12] Of note is that early start of csDMARD therapy is generally associated with a more beneficial disease outcome.[13-15] Nevertheless, in approximately a third of cases csDMARDs fail to control uveitis, and adding therapy with biologics - usually tumor necrosis factor alpha inhibitors - is required.[10,16-17,18-25] It is currently not possible to objectively predict the response to csDMARD in advance, thus, with the current treatment guidelines it may take a considerable amount of time for a group of patients to receive their optimal therapeutic regimen. All while being exposed to the visual-threatening risks of undertreatment or potential side effects of overtreatment. The early identification of patients in which csDMARDs will not sufficiently control eye inflammation will help mitigate these risks. Identification of these cases may be achieved by discovery of predictive immune-related protein signatures in cases that require biologics (csDMARD failure) versus cases that did not. Studies in patients with other inflammatory diseases have shown that blood protein profiling has the potential to predict treatment response before commencing treatment.[26] In this study, we performed immune-profiling of a cohort of children with non-infectious uveitis with ophthalmological follow-up to identify key proteomic profiles that can stratify response to csDMARDs in advance on a personalized basis.

## METHODS

**Patient and material collection**

The study was approved by the Medical Ethical Research Committee of the University Medical Center Utrecht in concordance with the Helsinki principles. Written informed consent was obtained from all patients ≥ 18 years, from both parents and patients in cases between 12-18 years of age, and from parents in cases < 12 years old. Peripheral blood samples (BD vacutainer® serum tubes) were obtained from patients with juvenile idiopathic arthritis-associated uveitis (JIA-U, n=14), idiopathic chronic anterior uveitis (i.e., no JIA) (CAU, n=7), (*HLA-B27*-positive) acute anterior uveitis (n=7), intermediate uveitis (IU, n=11), and panuveitis (PAN, n=33). Serum samples of 15 healthy controls (i.e., without a history of inflammatory eye disease) were collected from children without uveitis during surgery indicated for strabismus. Samples were collected at the outpatient department (uveitis biobank, n=48) or were the remainder of samples obtained for diagnostic purposes (diagnostic laboratory, n=39). All patients had active uveitis (new onset or relapse), and none of the patients received immunomodulatory treatment at the time of sampling. The diagnosis of uveitis was established by a trained uveitis specialist according to the SUN criteria.[27] All cases were recruited at the University Medical Center Utrecht, the Netherlands (tertiary referral center and *National Uveitis Center of Excellence*). All samples were obtained from patients with a diagnosis of non-infectious uveitis before the age of 18 years. Juvenile idiopathic arthritis was diagnosed according to the criteria of the *International League of Associations for Rheumatology*, or by former criteria (e.g., *European League Against Rheumatism*).[28,29] Patients with JIA were screened by an ophthalmologist according to the guidelines of the Academy of Pediatrics.[30,31] Patients with 1+ cells or more in the anterior chamber and treated with at least topical steroids were diagnosed with JIA-U. Patients using csDMARDs (n=37) were categorized into responders and non-responders. Patients were considered to be "responders" if inactive disease was achieved by csDMARD therapy (i.e., ≤1+ cells in aqueous humor and/or vitreous body after six months and with systemic corticosteroids ≤7.5 milligrams). Patients were considered to be "non-responders" if remission was not achieved under csDMARD therapy, and addition of a biological was indicated. This was defined as the presence of any of the following features:

1) ≥3+ cells in aqueous humor and/or vitreous body after four months

2) ≥2+ cells after 6 months and/or disease activity on fluorescein angiography

3) Necessity of use of systemic corticosteroids ≥ 7.5 milligrams/day).[32]

**Serum proteomic Olink analysis**

After blood withdrawal, serum tubes were kept for 30 minutes at room temperature and immediately centrifuged at 2000g for 10 minutes at room temperature. Serum was collected and stored directly at -80 °C. Frozen serum samples were shipped on dry ice to the Olink Facility (Uppsala, Sweden) without prior thawing and measured using the proximity extension assay (PEA) technology based on Proseek Multiplex panel (Olink Proteomics).[33,34] In short, PEA technology uses a set of antibodies that are linked with matching DNA-oligonucleotides per protein. After binding the protein, the oligonucleotides hybridize when brought into proximity and are extended by DNA polymerase, forming polymerase chain reaction (PCR) targets. Using next generation sequencing the PCR targets are quantified. The Olink *Explore 384 inflammation* panel was used to measure 368 proteins associated with inflammatory responses. The serum protein expression data from the Olink platform are expressed as an arbitrary unit (Normalized Protein eXpression [NPX]) representing the relative protein concentration based on a $\log_2$ scale. The full list of protein targets (n=368) is provided in **Supplementary table 1**.

**Data analysis and statistics**

Statistical analyses were performed in *R* (version 3.6.1). The statistical analysis of proteomic data was performed on protein expression data (NPX units). We used the *k*-nearest neighbours (kNN) algorithm to impute missing values (6.9%, interquartile range [IQR] 5.7-8.0) with *k*=5 with the R package *Hmsic*. Principal component analysis was performed using the *factoextra* package in R and batch effects (uveitis biobank versus diagnostic laboratory) were removed by the *ComBat* (Empirical Bayes) method implemented in the *sva* package in R (**Supplemental Figure 1**).[35,36] Differential expression analyses on batch-corrected data were conducted using a likelihood ratio test (LRT), and the proteins with a nominal *P value* <0.05 were considered differentially expressed proteins (DEP). Fisher's Exact Test or Pearson's Chi-square test were used to compare categorical variables between uveitis patients and controls, or between responders and non-responders. The Wilcoxon rank-sum test was used for continuous variables.

**Prediction of csDMARD response by machine learning models**

We built supervised machine learning models to predict response to csDMARDs (responders versus non-responders). Four machine learning models were built based on the random forest algorithm using the *randomForest R* package.[37] The first model was designed using ophthalmologic clinical data at diagnosis (model 1). 1) Age of uveitis onset, 2) sex, 3) laterality of uveitis, 4) maximum cell grade in the aqueous humor and/or vitreous body (depending on site of inflammation), 5) a complication at diagnosis (i.e., band keratopathy, posterior synechiae or cataract), 6) ANA seropositivity, 7) measurement of the central macular thickness in µm (CMT) on optical coherence tomography (OCT) imaging, and 8) measurement of retinal nerve fiber layer thickness (RNFL) in µm on OCT imaging were included as features (i.e., predictors), and the response to csDMARD was considered as output of the random forest model. The cell grade in the aqueous humor was scored according to the SUN criteria by an ophthalmologist specialized in pediatric uveitis.[27] The identical scale was applied for grading cells in the vitreous body (i.e., vitreous cell grade based on SUN criteria for aqueous humor scoring). In case of bilateral uveitis, the eye with the worst score was used for the model. Missing values in ANA seropositivity (n=1), measurement of CMT (n=6) and measurement of RNFL (n=13) were imputed using the *randomForest* package in R.[37] A second model was based on the full serum protein profile (n=368 proteins) of responders and non-responders (model 2). The ten most important features (*Feature importance metric*) were selected and used to run a third random forest model (model 3). The last model was a combination of model 1, the clinical parameters, and model 3, the 10-protein signature (model 4). The number of variables used at each split (mtry) with the lowest *out-of-bag error* was selected for each model (mtry = 3,19, 4, and 4, for model 1, 2 3, and 4 respectively). The number of trees was set at 5000 for all models. The performance of the models was evaluated using *leave-one-out* cross validation (i.e. the number of folds equals the number of samples, and for every fold, one sample is used as test set and the rest as the training set). The overall accuracy, true-positive rate (TPR) and true-negative rate (TNR) are evaluated based on the test set.

To establish the relative difference in risk profiles captured by the 10-protein signature, we stratified the 37 csDMARD patients into two clusters based on the first principal component of the expression profile of the ten proteins. To determine the optimal cutoff value we used the *surv_cutpoint* function of the *survminer* R package with a minimal proportion of observations of 0.4.[38] The cumulative hazard was plotted using

the *survival* R package and analyzed using the *coxph* and *ggforest* from the *survival* and *survminer* R package, respectively.[38,39]

## RESULTS

In total, 72 cases with pediatric uveitis were included in this study. Demographic characteristics of the study patients are shown in **Table 1.** No significant differences in sex and age were observed between uveitis patients and healthy controls. Targeted proteomics of serum was conducted in all cases and control samples. In total, 368 unique proteins were used for analysis. Comparison of the serum proteome of uveitis cases versus controls identified 62 differentially expressed proteins ($P$ <0.05), of which ARHGEF12 (Rho Guanine Nucleotide Exchange Factor 12), PRKAB1 (Protein Kinase AMP-Activated Non-Catalytic Subunit Beta 1), and DECR1 (2,4-dienoyl-CoA reductase) were the most differentially expressed (**Supplementary Table 2** and **Figure 1A**). However, the levels of these proteins varied substantially between cases regardless uveitis subtype (**Figure 1B**) suggestive for molecular heterogeneity linked to features beyond uveitis entity (i.e. treatment response).



**Table 1.** Demographics and baseline characteristics of the study patients (n=87).

| | Uveitis cases | Controls | *P-value* |
|---|---|---|---|
| N | 72 | 15 | |
| Male, n (%) | 26 (36) | 6 (40) | 0.78 |
| Age at sampling in years, median (IQR) | 13 (10-15) | 12 (5-27) | 0.87 |
| ANA seropositivity, n (%) | 35 (50) | N.A. | N.A. |
| Age at uveitis diagnosis in years, median (IQR) | 11 (8-14) | N.A. | N.A. |
| Duration of uveitis in years, median (IQR) | 0.13 (0.01-0.70) | N.A. | N.A. |

ANA, antinuclear antibody; IQR, interquartile range; N.A., not applicable.

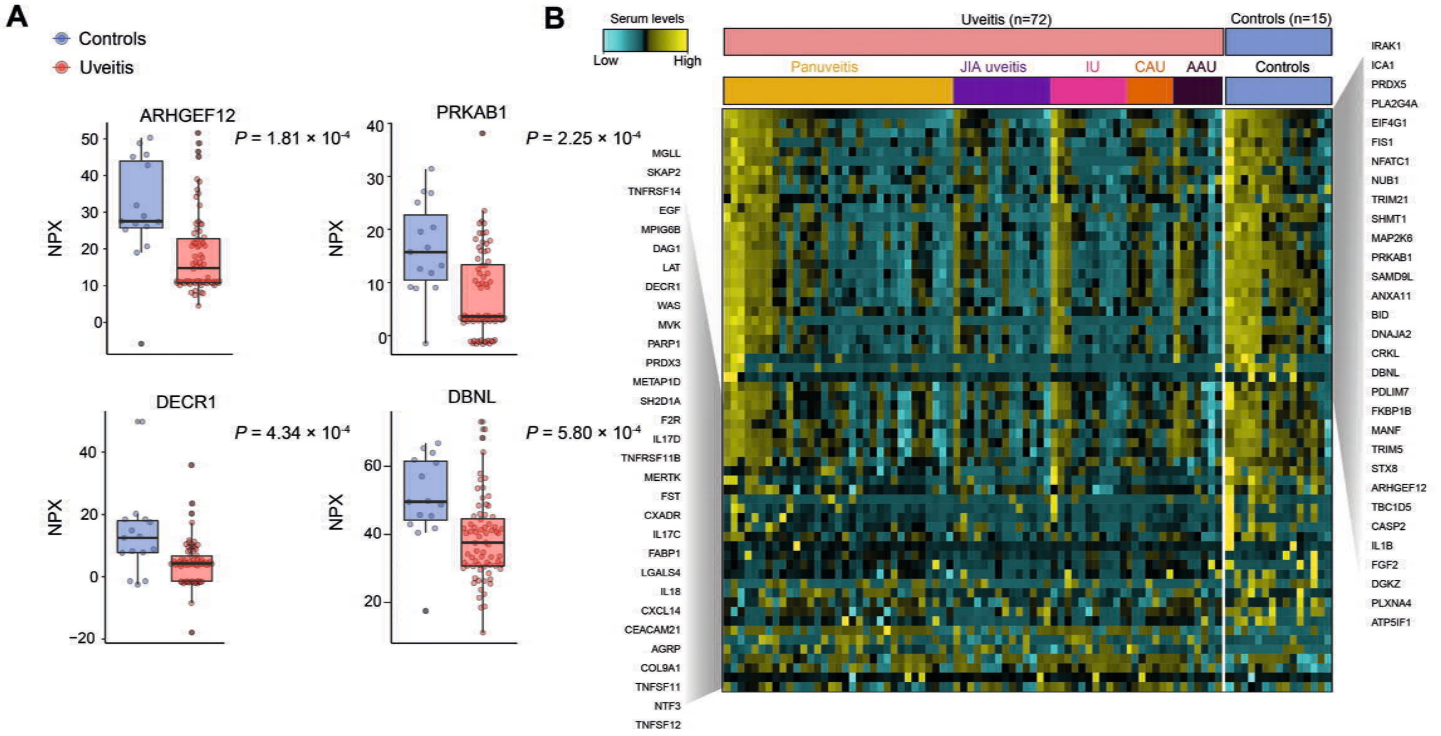


**Figure 1.** Serum protein analysis of pediatric uveitis (n=72) and healthy controls (n=15).

**A)** Scatter and boxplots of the top four most significantly different serum proteins between uveitis cases and healthy controls. Protein expression data and details on statistical analysis for each protein analyte (n=368) is shown in **Supplementary table 2. B)** Heatmap of the 62 differently expressed proteins between uveitis cases and controls. The levels for each protein analyte are shown for each of the samples in the study and are color-coded from low (in cyan) to high (in yellow). NPX, normalized protein expression unit from Olink array; ARHGEF12: Rho Guanine Nucleotide Exchange Factor 12; PRKAB1:Protein Kinase AMP-Activated Non-Catalytic Subunit Beta 1; DECR1: 2,4-Dienoyl-CoA Reductase 1; DBNL: Drebrin-like protein.

To explore this in more detail, we assessed the proteomic differences between csDMARD responders and non-responders. First, we filtered for patients that were sampled at diagnosis (46/72) and started csDMARD treatment after sampling (37/46). Of the 37 patients who started csDMARD during follow-up, 19/37 (51%) cases required anti-TNF-α therapy in addition to csDMARD for disease control, which we considered as "non-responders'' to csDMARD therapy (see Methods and **Figure 2A**). There was a moderate difference in the time to start with csDMARD between non-responders and the other 18 "csDMARD responders" (1 month versus 2.6 months, $P = 0.049$, **Table 2**). In addition, as expected, the cell grade in aqueous humor and/or vitreous body was higher in non-responders (1+ cells versus 3+ cells, $P = 0.003$). No other differences were observed between the two groups (**Table 2**). The proteomic profile of csDMARD responders versus non-responders was compared. The protein expression data of responders and non-responders is shown in **Supplementary table 3.** We built four machine learning models based on the random forest algorithm to predict csDMARD response using the clinical parameters at diagnosis, serum proteome (n=368), a top 10-protein signature (i.e. the ten most important features in the serum proteome random forest model) and a model using the clinical parameters and the top 10-protein signature. A 10-protein model showed an overall accuracy of 84% (95% confidence interval [CI] 68-94%), which was higher compared to a model based on the clinical parameters (**Figure 2B** and **Figure 2C).** The true-positive rate and true-negative rate of this model was 78% and 89%, respectively. Among the proteins, the top three proteins that drove this signature were osteomodulin (OMD), oncostatin M (OSM), and the plasmacytoid dendritic cell associated protein C-Type Lectin Domain Family 4 Member C (CLEC4C). Adding the top 10-protein signature to the model with clinical parameters improved the overall accuracy from 73% to 92%, indicating improvement of adding proteins into a classification model based on clinical features only (**Figure 2B**).

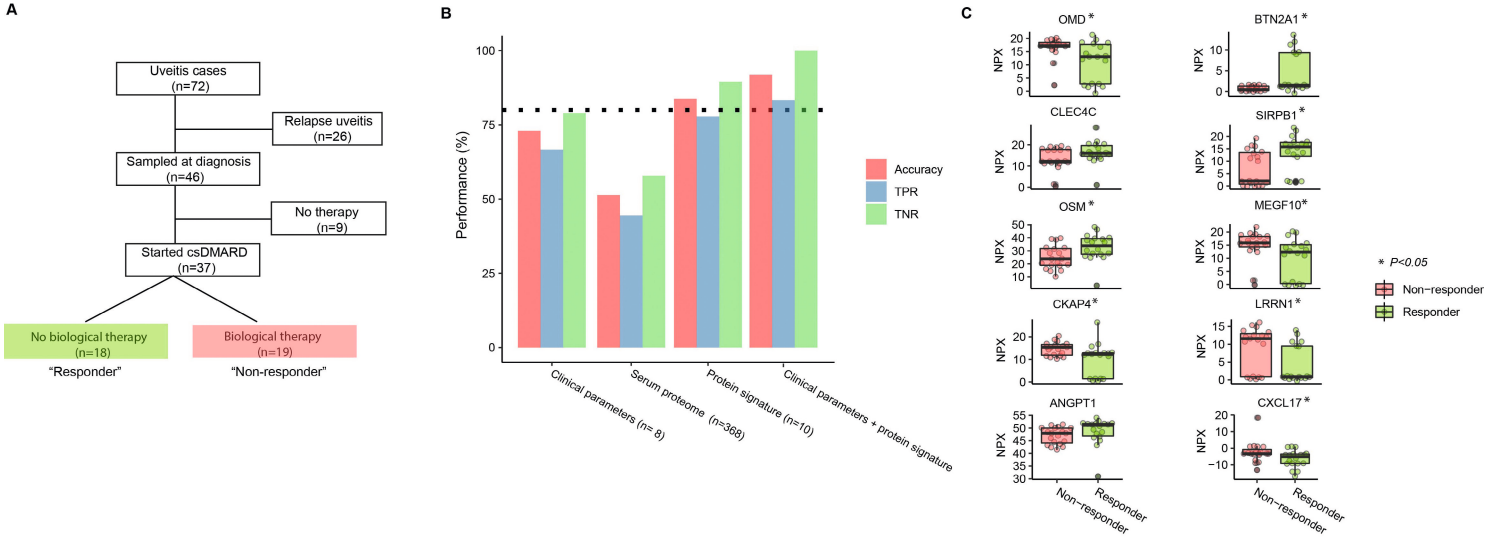


**Figure 2.** Random forest models performance for classification of csDMARD response in pediatric uveitis.

**A)** A flow chart indicating the selection of patients for analysis of csDMARD response at diagnosis (new onset uveitis). **B)** The accuracy, true negative rate and true positive rate of the random forest moddels based on eight clinical parameters (model 1), the serum proteome (model 2, n=368 proteins), the 10-protein signature (model 3) and the combination of clinical parameters and the 10-protein signature (model 4). The horizontal dotted line indicates a threshold accuracy of 80%. **C)** Boxplots of top ten most important proteins that distinguish responders from non-responders identified by random forest model 2. The proteins are sorted based on importance from left to right (feature importance metric). The asterisk indicates a $P<0.05$ from the likelihood ratio test between csDMARD responders and non-responders. TNR, true negative rate; TPR, true positive rate; NPX, normalized protein expression.

**Table 2.** Baseline and clinical characteristics at diagnosis of responders (n=18) and non-responders (n=19).

| | Responder | Non-responder | *P-value* |
|---|---|---|---|
| N (%) | 18 (49) | 19 (51) | |
| Male, n (%) | 4 (22) | 8 (42) | 0.20 |
| Age at uveitis onset in years, median (IQR) | 13 (11-15) | 10 (8-13) | 0.08 |
| Bilateral, n (%) | 16 (89) | 12 (63) | 0.12 |
| Location of uveitis | | | 0.80 |
| Anterior uveitis, n (%) | 5 (28) | 6 (32) | |
| Non anterior uveitis, n (%) | 13 (72) | 13 (68) | |
| Maximum cell grade aqueous humor and/or vitreous body, median (IQR) [a] | 1 (1-3) | 3 (3-4) | 0.003 |
| Measurement of central macular thickness in µm, median (IQR) | 296 (265-338) | 348 (266-399) | 0.17 |
| Measurement of retinal nerve fiber layer thickness in µm, median (IQR) | 193 (123-204) | 197 (168-219) | 0.32 |
| Complications, n (%) [b] | 9 (50) | 9 (47) | 0.87 |
| ANA seropositivity, n (%) | 6 (35) | 10 (53) | 0.24 |
| Type of csDMARD | | | 0.40 |
| Methotrexate, n (%) | 10 (53) | 11 (61) | |
| Mycophenolate mofetil, n (%) | 9 (47) | 7 (39) | |
| Time to start csDMARD in months, median (IQR) | 2.6 (0.7-3.4) | 1.0 (0.4-2.0) | $4.99 \times 10^{-2}$ |

ANA, antinuclear antibody; IQR, interquartile range; csDMARD, conventional synthetic disease modifying antirheumatic drugs.
[a] The cell grade in the aqueous humor and vitreous body was scored according to the SUN criteria.[27]
[b] Complications at diagnosis: band keratopathy, posterior synechiae or cataract.

To establish the relative difference in risk profiles captured by this 10-protein signature, we split the 37 csDMARD patients into two clusters based on the levels of the ten proteins (using the first principal component of the expression data) (**Figure 3A** and **Supplementary table 4**). Cluster 1 consisted primarily of non-responders (15/17), and cluster 2 mainly of responders (16/20). Survival analysis showed that cases stratified upon the 10-protein signature differ significantly in their probability for csDMARD failure (*log rank test = 2.28 x* $10^{-5}$). At nine months after diagnosis, half of the cases in cluster 1 failed on a csDMARD compared to 15% cases in cluster 2 (**Figure 3B**). Multivariate Cox proportional hazard analysis adjusting for age, sex, anatomical location of uveitis and cell grade in aqueous humor and/or vitreous body (as a proxy for uveitis activity) revealed that patients in cluster 1 (n=17) had an substantially increased risk for being a "non-responder" compared to cases in cluster 2 (n=20) (hazard ratio [95% confidence interval] = 12.8 [2.5-64.6], *P* = 0.002) (**Figure 3C**). These data indicate that a proteomic profile can be harvested to predict risk categories for csDMARD failure in patients with pediatric uveitis.

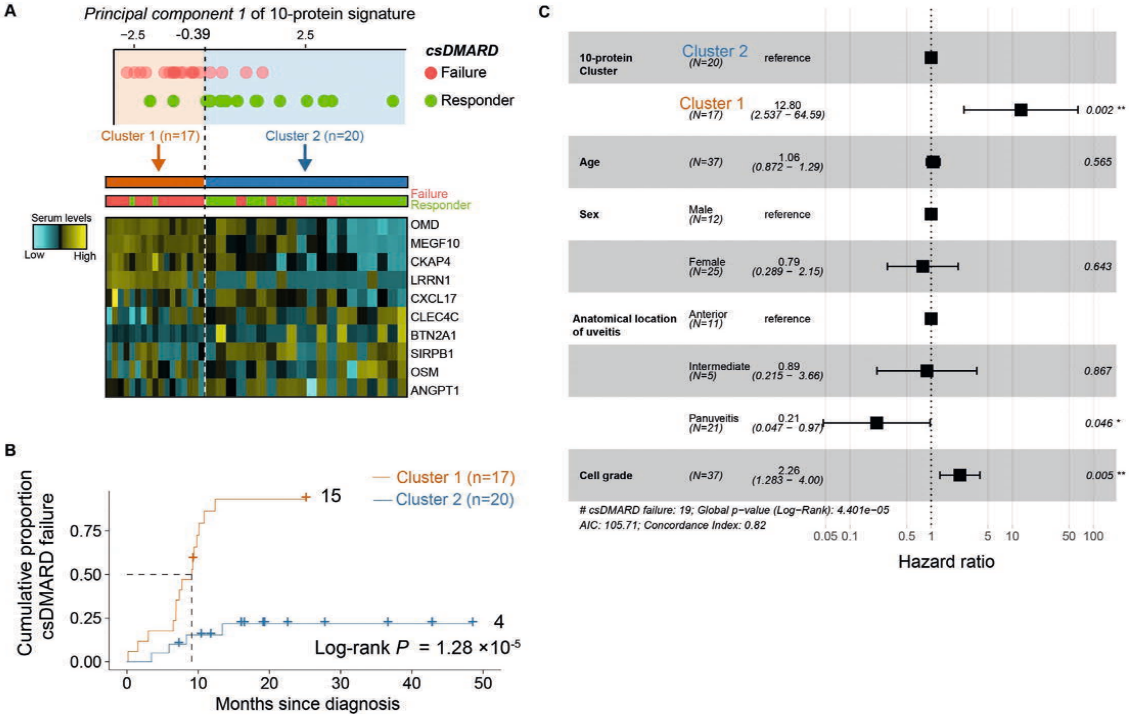


**Figure 3.** A 10-protein signature stratifies cases with high risk for csDMARD failure at pediatric uveitis diagnosis.

**A)** Two clusters are identified based on the first principal component of the expression of the ten serum proteins identified by random forest model 2 (dotted line). The relative levels of the 10 serum proteins for each case in both clusters is color-coded from low (cyan) to high (yellow). **B)** The cumulative event curve for csDMARD failure for each of the two 10-protein clusters identified in *a*. The dotted line indicates the time to 50% csDMARD failure in Cluster 1. **C)** A forest plot of the multivariate Cox model used to assess the proportional hazard for csDMARD failure for each cluster identified in *a*. hazard was adjusted for age, sex, anatomical location of uveitis and cell grade in the aqueous humor and/or vitreous body.

## DISCUSSION

Here we used targeted proteomic profiling of serum samples drawn from children with active non-infectious uveitis in order to identify a protein signature by diagnosis that stratifies cases that have a high risk for csDMARD failure during follow up. These results show that a 10-protein signature was highly predictive for csDMARD failure in children with non-infectious uveitis. The identified protein signature will guide us to the realization of personalized medicine for children with non-infectious uveitis.

Treatment guidelines for pediatric uveitis, except for JIA-uveitis, are sparse and heavily dependent on individual clinical monitoring of disease.[18,22-25] This is in part due to the limited number of clinical trials in this patient population and the lack of biomarker studies for the prediction of treatment response.[19-21] Ideally, clinical-decision tools (i.e., algorithms) integrate clinical parameters with biological data to better predict the best treatment option, which considers prompt disease control, the least adverse drug effects, and the possibility to reduce the disease burden. Here, we provide a proof of concept for stratifying the response to csDMARD in children with non-infectious uveitis using a blood protein signature.

There are however a number of considerations and limitations of the current study, which we outline in detail. In our cohort, half of cases had an indication for anti-TNF-α therapy in addition to a csDMARD, which is higher than reported in literature (~30%).[10,16,17] However, patients referred to a tertiary center hospital, as in our study, are 1.6 times more likely to be treated with biological therapy when compared to patients referred to primary care, which may explain the higher rate.[40] In addition, biological therapy can also be initiated to prevent long-term use of systemic corticosteroids. The high number of patients with refractory uveitis and the beneficial therapeutic effect of biologics in these cases emphasize the unmet need for early identification of csDMARD failure.[19,20,21,41] A parsimonious model based on the ten most discriminative proteins showed the highest overall accuracy. Patients stratified by the expression levels of these ten proteins showed a large difference in risk for csDMARD failure. Although the proportion of each uveitis subtype in our cohort did not reflect the population at larger (i.e., anterior uveitis is by far the most common type seen in clinics), our analysis revealed that the protein signature stratified patients independent of anatomical location of uveitis. We therefore consider that these results are applicable to the wider population of non-infectious uveitis cases.

Although we observed influence of panuveitis and treatment response to csDMARD as an interacting covariate in a multivariate Cox model (including also age, sex and cell grade) (**Figure 3C**), Cox proportional analysis assessing the overall relationship between anatomical location of uveitis and csDMARD response did not support an association with panuveitis ($P$ = 0.76). Also, the median time to csDMARD was shorter in non-responders compared to responders (1 month versus 2.6 months). When adding the time to csDMARD as a covariate to the Cox model (Cox proportional hazard analysis adjusting for age, sex, anatomical location, cell grade, and time to csDMARD), we found the predictive capacity of the serum 10-protein signature to be modestly affected (Cox $P_{\text{10-protein+age+sex+location+cell grade}} = 0.002$, Cox $P_{\text{10-protein+age+sex+location+cell grade+time-to csDMARD}} = 0.08$), which supports that the 10-protein signature has clinical potential to predict csDMARD response. Four out of 19 non-responders (21%) were clustered with responders and illustrates that, although a vast improvement over current state of the art (i.e., unable to predict csDMARD response), our algorithm is not perfect and requires prospective validation to determine the precise accuracy and robustness in a 'real-life' setting. Regardless, it is of interest to note that this signature contained proteins linked to uveitis biology and treatment response in other inflammatory diseases. For example, oncostanin (OSM) is reported as a biomarker for treatment response in patients with inflammatory bowel disease and CLEC4C is a hallmark protein of plasmacytoid dendritic cells that are implicated in non-infectious uveitis.[42-43]



Although we determined that the 10-protein signature was found to be higher than the clinical model, we would like to emphasize that the model based on eight clinical parameters was also relatively accurate for prediction of csDMARD failure. These eight clinical parameters are also part of the now widely used SUN criteria for non-infectious uveitis assessment. However, we deliberately left out posterior segment imaging such as fluorescein angiography scores, because these imaging data would not be available for the majority of patients with anterior uveitis. The lack of an objective disease severity marker available across all type of non-infectious uveitis, makes predictive modelling challenging. To overcome this, we considered taking a parameter with a relatively similar context of information that is available for most cases (vitreous haze was not scored in all patients). In our opinion "number of cells" at diagnosis may serve as a solution to this problem where we used the highest cell score in either aqueous or vitreous fluid to be able to conduct analyses for all cases of non-infectious uveitis. Consequently, for anterior uveitis, this means that the disease severity was scored according to the SUN criteria (i.e. number of cells in the

anterior chamber), while vitreous cell score for non-anterior uveitis is deviating from these criteria.[27] Regardless, we demonstrate that this parameter (i.e., the maximum cell grade in the anterior chamber and/or vitreous body at diagnosis) was the most discriminative clinical feature for csDMARD response according to random forest analysis. Also, we noted a relationship between csDMARD response and cell grade using multivariate Cox proportional hazard analysis (**Figure 3C**). It is tempting to speculate that a higher intraocular cell grade reflects a high disease activity and severity, which requires more often biological therapy to control inflammation. These results underscore that the standardized SUN criteria can stratify patients at high risk for csDMARD failure. In this study, however, we extend this and show proof of concept that blood biomarkers can further (i.e., independently) improve the prediction of csDMARD failure at diagnosis. A model based on the clinical parameters and the 10-protein signature improved the detection of csDMARD failure from 73% to 92% and this supports that adding molecular biomarkers in blood can significantly improve early detection of treatment failure in pediatric uveitis. Future studies should aim to integrate a wider variety of clinical parameters with precise cut-off levels for the protein biomarkers to generate a clinical decision algorithm for the stratification of patients with a high risk for csDMARD failure.

In conclusion, we showed that a proteomic signature detectable at diagnosis could accurately stratify csDMARD response. Future studies should be focusing on the clinical implication of the use of proteomics in combination with the clinical observations in predicting treatment response and understanding the mechanism of how different patients respond to immunosuppressive therapy, paving the path towards personalized medicine.

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# SUPPLEMENTARY DATA

## Supplementary tables

**Supplementary table** 1: Full list of protein targets of the Olink Explore 384 inflammation panel (n=368).

| Protein | | | | |
|---|---|---|---|---|
| ACTN4 | CCL24 | CNTNAP2 | EIF5A | HLA-DRA |
| ADA | CCL25 | COL9A1 | ENAH | HLA-E |
| ADAM23 | CCL26 | COLEC12 | ENPP5 | HPCAL1 |
| ADGRE2 | CCL28 | CRELD2 | ENPP7 | HSD11B1 |
| AGER | CCL3 | CRHBP | EPCAM | HSPA1A |
| AGRN | CCL4 | CRIM1 | EPHA1 | ICA1 |
| AGRP | CCL7 | CRKL | EPO | ICAM4 |
| ALDH3A1 | CCN2 | CRLF1 | ERBB3 | IDS |
| AMBN | CD160 | CSF1 | ESM1 | IFNG |
| AMN | CD200 | CSF3 | F2R | IFNGR1 |
| ANGPT1 | CD200R1 | CST7 | FABP1 | IFNLR1 |
| ANGPTL2 | CD22 | CTRC | FABP9 | IKBKG |
| ANGPTL4 | CD244 | CTSC | FASLG | IL10 |
| ANXA11 | CD276 | CTSO | FCAR | IL10RA |
| AOC1 | CD4 | CXADR | FCRL2 | IL10RB |
| ARHGEF12 | CD40 | CXCL1 | FCRL3 | IL11 |
| ARNT | CD40LG | CXCL10 | FCRL6 | IL12B |
| ARTN | CD48 | CXCL12 | FGF19 | IL12RB1 |
| ATP5IF1 | CD58 | CXCL14 | FGF2 | IL13 |
| AXIN1 | CD6 | CXCL17 | FGF5 | IL15 |
| B4GALT1 | CD70 | CXCL3 | FIS1 | IL15RA |
| BACH1 | CD79B | CXCL6 | FKBP1B | IL16 |
| BANK1 | CD83 | CXCL8 | FLT3LG | IL17A |
| BCL2L11 | CD84 | CXCL9 | FOXO1 | IL17C |
| BCR | CDON | DAG1 | FST | IL17D |
| BID | CDSN | DAPP1 | FSTL3 | IL17F |
| BSG | CEACAM21 | DBNL | FXYD5 | IL17RB |
| BTN2A1 | CEP164 | DECR1 | GAL | IL18 |
| BTN3A2 | CHRDL1 | DFFA | GALNT3 | IL18R1 |
| C1QA | CKAP4 | DGKZ | GBP2 | IL1A |
| CASP2 | CKMT1A_CKMT1B | DNAJA2 | GLOD4 | IL1B |
| CCL11 | CLEC4A | DNER | GMPR | IL1R2 |
| CCL13 | CLEC4C | DNPH1 | GOPC | IL1RL2 |
| CCL17 | CLEC4D | DPP10 | GZMA | IL1RN |
| CCL20 | CLEC4G | EDAR | GZMB | IL2 |
| CCL21 | CLEC7A | EGF | HCLS1 | IL20 |
| CCL22 | CLIP2 | EGLN1 | HEXIM1 | IL20RA |
| CCL23 | CLSTN2 | EIF4G1 | HGF | IL22RA1 |

IL24
IL2RB
IL32
IL33
IL3RA
IL4
IL4R
IL5
IL5RA
IL6
IL7
IRAK1
IRAK4
ISM1
ITGA11
ITGA6
ITGB6
ITM2A
JCHAIN
JUN
KLRB1
KLRD1
KRT19
KYNU
LAIR1
LAMA4
LAMP3
LAP3
LAT
LGALS4
LGALS9
LGMN
LHPP
LIFR
LILRB4
LRRN1
LSP1
LTA
LTBR
LTO1
LY6D
LY75
LY9
MANF
MAP2K6
MAPK9
MATN2
MEGF10
MEPE
MERTK
METAP1D
MGLL
MGMT
MICB_MICA
MILR1
MLN
MMP1
MMP10
MPIG6B
MVK
MYO9B
MZB1
NBN
NCF2
NCK2
NCLN
NCR1
NELL2
NFASC
NFATC1
NFATC3
NME3
NPPC
NRTN
NT5C3A
NTF3
NUB1
NUDC
OMD
OSCAR
OSM
PADI2
PAPPA
PARP1
PCDH1
PDGFB
PDLIM7
PGF
PIK3AP1
PKLR
PLA2G4A
PLAUR
PLXNA4
PNLIPRP2
PNPT1
PON3
PPP1R9B
PRDX3
PRDX5
PREB
PRELP
PRKAB1
PRKCQ
PROK1
PRSS8
PSIP1
PSMG3
PSPN
PTH1R
PTPN6
PTPRM
PTX3
RAB37
RAB6A
RABGAP1L
REG4
RGS8
ROBO1
SAMD9L
SCG3
SCGB1A1
SCGB3A2
SCGN
SCRN1
SELPLG
SERPINB8
SH2D1A
SHMT1
SIGLEC1
SIGLEC10
SIRPB1
SIT1
SKAP2
SLAMF1
SLAMF7
SLC39A5
SMOC2
SMPDL3A
SPINK4
SPINT2
SPON1
SPRY2
SRPK2
STX8
SULT2A1
TANK
TBC1D5
TFF2
TGFA
TGFB1
TIMP3
TLR3
TNF
TNFAIP8
TNFRSF11A
TNFRSF11B
TNFRSF13B
TNFRSF13C
TNFRSF14
TNFRSF4
TNFSF10
TNFSF11
TNFSF12
TNFSF13
TPP1
TPSAB1
TPT1
TRAF2
TREM2
TRIM21
TRIM5
VASH1
VEGFA
VEGFD
WAS
WFIKKN2
WNT9A
YTHDF3

**Supplementary table 2:** Expression data of controls (n=15) and uveitis patients (n=72). Normalized Protein expression (median [min-max]).

| Protein | Controls | Uveitis | pvalue |
|---|---|---|---|
| ARHGEF12 | 27.6(-5.8-50.3) | 14.8(4.5-51.6) | 0.000181168066607188 |
| PRKAB1 | 15.7(-1.5-31.4) | 3.6(-1.6-38.1) | 0.000225282593928966 |
| DECR1 | 12.5(-2.5-49.7) | 4.3(-17.8-35.8) | 0.000434556577974314 |
| DBNL | 49.5(17.5-66.8) | 37.6(11.1-73.1) | 0.00057986929289534 |
| LGALS4 | 8.2(-2.2-18.1) | 2(-14.4-13.7) | 0.00112532531171855 |
| PDLIM7 | 53.3(14.5-73.5) | 36.8(12.3-81.1) | 0.00176764448578023 |
| FGF2 | 13.4(-0.6-27.5) | 0.8(-0.7-28.8) | 0.00182562139614398 |
| CRKL | 46.6(10.8-71.2) | 33.8(5.8-71.2) | 0.0021720143853353 |
| TRIM21 | 17.3(-0.6-41.6) | 10.9(-20.8-42.8) | 0.00278413569907215 |
| WAS | 9.5(-1.2-24.2) | 1.3(-10.5-28.9) | 0.003102448241829 |
| IRAK1 | 22.5(9-38) | 17.6(-1.6-38.3) | 0.0033788582288907 |
| TNFSF11 | 9.7(-11.4-24.9) | 16.6(-0.2-28.1) | 0.00353592088510299 |
| IL1B | 0.4(-0.3-27.4) | 0.3(-1-24.5) | 0.00406988400899025 |
| FKBP1B | 27.3(3.7-47.8) | 15.3(-2.5-57.8) | 0.0042469211472365 |
| PRDX5 | 34.1(19.3-45.5) | 25.3(5.3-45.1) | 0.00466617687509189 |
| CASP2 | 25.3(-3-48.5) | 15.8(-1.9-53.2) | 0.00488621965307355 |
| DNAJA2 | 50.2(17.2-67.6) | 39.8(22-71.1) | 0.00500082195188113 |
| MANF | 56.3(27.9-86.7) | 45.6(30.1-77.4) | 0.00511795258633647 |
| MAP2K6 | 47(-0.7-60.9) | 34.5(18.9-68.3) | 0.00581106389818831 |
| STX8 | 12.7(-1.7-28.7) | 0.7(-1.6-34.3) | 0.00700380131569844 |
| ANXA11 | 24.4(-2-45.7) | 15(-2.3-43.7) | 0.00858561904158526 |
| EGF | 65.6(44.8-75.7) | 61.6(41.3-75.2) | 0.0104410952739598 |
| SHMT1 | 11.1(-3.1-27.6) | 2.4(-15-31.7) | 0.0109653632326166 |
| LAT | 29.7(22.9-50.1) | 27.6(17.9-37.3) | 0.0111924621881298 |
| CXADR | 10.8(-0.2-27.7) | 1.3(-0.3-25) | 0.0116538380898611 |
| DGKZ | 0.5(-0.1-12.7) | 0.4(-0.7-12.6) | 0.0119722364655875 |
| IL18 | 0.1(-0.8-15) | 0.7(-13.4-20.6) | 0.0120161783802461 |
| SH2D1A | -0.2(-1.3-40.8) | 2.4(-1.2-21.1) | 0.0121887055295717 |
| TRIM5 | 18.8(-3.1-37.1) | 13.8(-3.2-44.9) | 0.0131047859986264 |
| MGLL | 26.5(9.7-59.2) | 21(-1.9-47.8) | 0.0137329450941404 |
| FST | -0.1(-1.6-19.7) | 0.3(-14.2-15.9) | 0.0151923051717592 |
| TBC1D5 | 29.3(0.1-53.3) | 22.8(11.3-58.9) | 0.0154737030255973 |
| FIS1 | 11.1(-0.3-59.3) | 0.6(-0.7-39.5) | 0.0167252844062452 |
| BID | 25(1.2-43.7) | 16.5(-1.6-52.7) | 0.0186658510091934 |
| SKAP2 | 60.8(42.6-78) | 53.9(15.4-83.8) | 0.0186699006273013 |
| CXCL14 | -11.1(-20-1) | -1.7(-16.7-25.6) | 0.0189611638443406 |
| F2R | -16.8(-24.2-26.9) | -16.4(-29.3--4.2) | 0.019705236228336 |
| PRDX3 | 0.4(-0.9-30.2) | 0.2(-0.8-21.7) | 0.0197127076488156 |
| NFATC1 | 23.7(6.4-49) | 20.2(5.8-52.6) | 0.0201537911435247 |
| MPIG6B | 49.1(32.4-55.9) | 45.2(21.3-58.2) | 0.0213138681442998 |
| DAG1 | 23.3(-0.6-28.9) | 18.2(-0.1-30.4) | 0.0214027630658318 |

Supplementary table 2: Continued

| Protein | Controls | Uveitis | pvalue |
|---|---|---|---|
| FABP1 | -1.2(-16.1-35.4) | -1.3(-24.3-23.6) | 0.021478383685544 |
| IL17C | -2.9(-4.4-26.2) | -3.1(-16.7-10.5) | 0.0222822578371256 |
| MVK | 9.4(-2.5-27.6) | 7.3(-12.5-22.4) | 0.0261928649850133 |
| SAMD9L | 30.7(12.6-58.4) | 27.8(14.1-54.1) | 0.0271091842153963 |
| PLXNA4 | 29.2(18-58.2) | 27.2(-0.2-48.5) | 0.0289957725626253 |
| COL9A1 | 83.9(6.1-93.9) | 83.2(5-100.4) | 0.0290582564968896 |
| METAP1D | 0.2(-0.8-40) | 0.1(-1-26.4) | 0.0296585634752144 |
| PLA2G4A | 11.8(-2.3-49.8) | 4.8(-2.6-37.5) | 0.0321379704537506 |
| AGRP | -0.4(-1.2-9.6) | 2.5(-9.8-23) | 0.0327986386781322 |
| NUB1 | 22.7(-2.4-52.1) | 14.2(-2.6-41.6) | 0.035202189172653 |
| TNFRSF11B | 0(-0.6-19.6) | 0.3(-0.3-8.1) | 0.0383531163055802 |
| ICA1 | 15.8(-1.8-35.1) | 12(-3-38.7) | 0.0385723259531182 |
| IL17D | 0.1(-0.4-20.7) | 0.3(-0.4-20.6) | 0.0388942121555556 |
| TNFSF12 | 15.7(9.7-21.5) | 17.5(11.2-23.1) | 0.0396506918809212 |
| ATP5IF1 | 32(15.8-72.7) | 26.5(-0.9-51.2) | 0.0398742392309195 |
| EIF4G1 | 36.4(-0.7-62.9) | 23.8(-0.3-66.2) | 0.0416777670003473 |
| TNFRSF14 | 18.3(2.4-24.3) | 15.6(2.4-22.3) | 0.0444604318743775 |
| MERTK | 0.2(0-14.3) | 0.6(-0.3-9.9) | 0.0446228271867273 |
| CEACAM21 | -0.1(-12.8-20.8) | 0.2(-13.8-30.3) | 0.046161702737423 |
| NTF3 | 0(-18-0.9) | 0.4(-13.2-11.7) | 0.0469218807586243 |
| PARP1 | 0.8(0-57.6) | 0.3(-0.4-36.7) | 0.0479573559328946 |
| CXCL6 | 35.7(4.6-49.7) | 39.4(25.2-56.5) | 0.0512745334754435 |
| CTSO | 0.1(-0.9-12.9) | 1.6(-0.1-17.2) | 0.0533534730290941 |
| BANK1 | 35(5-61.1) | 28.3(11.6-67.5) | 0.0553014336085174 |
| LY6D | 0.4(-0.4-18.4) | 0.5(-0.7-14.5) | 0.0557719377258581 |
| CD200 | 9.2(-0.2-16.5) | 1.5(-0.1-17.7) | 0.0579391767724711 |
| CXCL8 | 21.4(-0.7-46.6) | 16.8(-0.5-65.9) | 0.0594697584081581 |
| NT5C3A | 31.9(8.1-59.6) | 22.6(-4-69.2) | 0.0615049861675502 |
| LAP3 | 0.8(-0.1-14.3) | 0.3(-11.3-18.8) | 0.0622160562951997 |
| CKMT1A_CKMT1B | -0.8(-1.5-29.7) | -0.4(-41.8-24.4) | 0.0631404241227632 |
| PPP1R9B | 28(17-58.4) | 23.7(-4.6-53.7) | 0.0633828174749292 |
| VASH1 | -0.1(-0.4-23.1) | 0.1(-0.6-1.4) | 0.0732162127520941 |
| PGF | 0.6(0.2-1) | 0.7(-0.2-23.2) | 0.0784724752410019 |
| CD40 | 14.4(0.3-40.9) | 12.4(0.3-23.3) | 0.0784946502957538 |
| HPCAL1 | 19.3(0-48.4) | 15.8(0-36.7) | 0.0800657809348207 |
| MEGF10 | 1.5(-0.1-19.9) | 14.5(-0.4-23.1) | 0.0803140196192225 |
| CRIM1 | 0.4(0.2-11.8) | 1.1(0.1-15.8) | 0.0825435128719881 |
| HSPA1A | 13.8(-1.8-44.3) | 3.8(-2.3-40.9) | 0.0828151493644847 |
| CLIP2 | 31.8(-1.9-70) | 21.9(-1.4-62.7) | 0.0921645738997159 |
| AGRN | 0.6(-0.1-16.3) | 0.9(-0.3-16.6) | 0.0926189455045467 |
| CTSC | 15.6(0.5-34.6) | 12.5(-0.2-30.9) | 0.0969546574112677 |

Supplementary table 2: Continued

| Protein | Controls | Uveitis | pvalue |
|---|---|---|---|
| IL4R | 11.2(-0.3-23.7) | 5.6(0-17.5) | 0.0984829634571075 |
| SCRN1 | 1.8(1.2-28.3) | 1.3(-10.7-33.1) | 0.0990352759106804 |
| AXIN1 | 24.6(-5.2-53.8) | 19.1(6.2-53.5) | 0.102859859498 76 |
| IFNGR1 | 0.3(-0.2-8.8) | 0.5(0-8.8) | 0.105660936662091 |
| CD58 | 0.4(0.2-8.5) | 0.5(-0.5-1.3) | 0.106115720896452 |
| C1QA | 0(-0.7-0.6) | 0.2(-0.4-0.7) | 0.107790722463992 |
| DNER | 0.2(-0.7-13.6) | 1.5(-0.6-15.9) | 0.108667376390479 |
| IDS | 0.2(-0.2-0.4) | 0.2(-0.3-0.4) | 0.110845561579582 |
| DAPP1 | -0.3(-1.5-47.8) | 0.4(-16.1-19.8) | 0.110872733478641 |
| TPSAB1 | -1.6(-21.2-12.6) | -0.9(-20.3-11.7) | 0.111074296335711 |
| IRAK4 | 32.3(-4.4-59.6) | 19.4(-4.2-65.5) | 0.111726827833456 |
| LIFR | 0.8(0.3-11.3) | 10.6(0.3-16.2) | 0.111820267163302 |
| SERPINB8 | 11.5(-2.8-36.3) | 7.6(-8.8-27.4) | 0.11226555315071 |
| CDON | 9.4(-1.3-20.1) | 12.7(-1.4-21.4) | 0.112392700394624 |
| ALDH3A1 | 9.9(-1.1-32.7) | 2.3(-1.3-33.8) | 0.115204813055147 |
| IL12B | 0.8(-0.7-26) | 13.9(-10.5-29.6) | 0.117979971179307 |
| SPON1 | 0.1(-0.5-10.2) | 1.2(-0.4-17.8) | 0.123661403037483 |
| CCL3 | 15.1(0.7-42.4) | 13(-1.9-31.6) | 0.125751949203504 |
| LGALS9 | 0.5(-0.3-18.3) | 0.6(-0.2-13.8) | 0.126783848202112 |
| IL7 | 32(17-48.4) | 33.6(18.5-49.7) | 0.129863498285118 |
| TNFSF13 | 3.7(3.1-18.3) | 12.5(-3.6-21) | 0.146387927170279 |
| MYO9B | 0(-0.3-28.5) | 1.4(-1.5-38.7) | 0.146685628108004 |
| PON3 | 0.2(-16.4-0.8) | 0.3(-12-1) | 0.150377151945978 |
| FABP9 | 0.5(-0.6-16.2) | 1.6(-0.7-25.2) | 0.154870150507694 |
| TNFRSF13B | 0.5(0-15.3) | 0.6(-0.3-16.6) | 0.159081592254679 |
| REG4 | 0.5(-0.1-14.7) | 0.4(-0.7-13.8) | 0.171120019324619 |
| CXCL9 | 0.3(-0.6-17.1) | 0.3(-15.8-30.7) | 0.171738562438789 |
| DFFA | 22(8.6-48.6) | 20.3(5.6-44.7) | 0.174193247464681 |
| ANGPTL2 | -0.8(-1.6-12.3) | 2.1(-17.6-14.4) | 0.175730740503805 |
| EPHA1 | -0.3(-1.5-12.3) | 0(-9.9-1.2) | 0.176493109286109 |
| CKAP4 | 11.8(-0.5-22.5) | 12.7(0.3-26.4) | 0.183322662707233 |
| SULT2A1 | 0(-0.8-30.2) | 0(-1.2-17.3) | 0.184644814368591 |
| OMD | 16.2(-2.3-21.2) | 16.1(-1.3-23.2) | 0.184895023419368 |
| FCRL2 | 0.4(-1-13.4) | 0.6(-0.7-19.1) | 0.188107831774337 |
| CD79B | 13.7(-2.2-31) | 16.9(-1.8-31.3) | 0.19296479074328 |
| ACTN4 | 0.2(-0.3-18.3) | 0(-0.6-13.8) | 0.193417155712608 |
| TNFSF10 | -0.1(-0.6-11.8) | 3.1(-0.4-18.8) | 0.198797507577648 |
| PTPRM | 0.8(0.4-18.6) | 11.4(0.3-17.2) | 0.199458406134104 |
| CRELD2 | 14.5(0.7-29.6) | 12.7(0.1-26.3) | 0.20033209277523 |
| CXCL10 | 2.3(1.5-18.3) | 13.1(-1.1-32.4) | 0.203721756583347 |
| GLOD4 | 15.9(-2.2-32.4) | 11.3(-2.9-34.3) | 0.208288069862761 |

Supplementary table 2: Continued

| Protein | Controls | Uveitis | pvalue |
|---|---|---|---|
| IL18R1 | 0.5(0.2-11) | 0.7(-0.3-15.3) | 0.2095730068808 |
| CD84 | 14.5(10-18.8) | 14.5(4.3-20.6) | 0.214927325665034 |
| NCK2 | -0.1(-0.8-49.3) | 1.5(-0.9-38.5) | 0.224133821485014 |
| RGS8 | 0.1(-11.5-27.8) | -0.1(-1.1-23.8) | 0.22597026135576 |
| HEXIM1 | 14(-5.6-43.9) | 9(-5.6-46.1) | 0.236809613973709 |
| TNFRSF13C | 11.4(-1.3-38.1) | 9.5(-0.6-28.6) | 0.242447008976723 |
| IL33 | 1(-0.7-30.7) | 0.3(-12.3-13.5) | 0.243080782380167 |
| CCL26 | 28.2(14.3-43.4) | 30(1.7-53.7) | 0.244125642427561 |
| SPRY2 | -0.2(-0.9-31.5) | 2.7(-1-24.1) | 0.245181019549037 |
| SPINT2 | 19.4(3.7-28.5) | 17.3(-8.1-27.3) | 0.248719388344054 |
| MGMT | 30.6(-2.5-75.1) | 21.6(-2.4-75.4) | 0.250483639283708 |
| BCR | 15(-3.3-48.2) | 6.3(-3.5-43.4) | 0.252820572545711 |
| PDGFB | 58(49.4-60.8) | 58.5(49-64.9) | 0.254884802131499 |
| PSMG3 | 14.1(6.4-48.4) | 15.1(-3.9-42.5) | 0.260883742690943 |
| PCDH1 | 0.4(0.2-0.6) | 0.5(-0.2-8.7) | 0.261286146550288 |
| MATN2 | 10.5(-1.1-23.6) | 13.2(-1.1-22.9) | 0.263474532167877 |
| VEGFD | 0.1(-0.5-0.7) | 0.4(-11.4-12.7) | 0.264150657301606 |
| IFNG | 0.4(-15.3-20.4) | 0.7(-21.9-55.4) | 0.264814189803668 |
| ENPP7 | -1.2(-22.2-27.7) | -0.3(-23.3-12.4) | 0.268335430643813 |
| CXCL3 | 47.2(31-77.9) | 44.2(25.9-73.3) | 0.271845263597834 |
| TPT1 | 0.6(-11.1-1.2) | -0.1(-15.4-19.6) | 0.279649060050106 |
| B4GALT1 | 0.5(-0.3-14.7) | 0.5(-0.2-12.3) | 0.280500240880193 |
| MZB1 | 0.7(-0.9-18.9) | 0.7(-0.8-25) | 0.284459721823263 |
| ARTN | -0.1(-0.5-0.6) | 0.1(-0.5-14.6) | 0.28471904238216 |
| CCL25 | 0.5(0-19.1) | 0.5(-0.4-18.4) | 0.28667740435313 |
| CD244 | 11.1(-0.5-19.7) | 9.9(-0.4-17.7) | 0.290122135171886 |
| RAB37 | 1.4(0.8-25.3) | 10.4(-2.7-64.6) | 0.293290397689407 |
| CNTNAP2 | 17(1.3-39.1) | 19.2(-2.2-36) | 0.293862751779304 |
| TREM2 | 0.8(-0.2-17.2) | 0.1(-12.3-13.2) | 0.295563013971251 |
| PNPT1 | 0.5(-0.5-28.6) | 0.4(-18.2-74.8) | 0.2970583459147 |
| NME3 | 0(-0.3-10.2) | 1.6(-0.2-15.9) | 0.297952852658202 |
| JUN | -0.9(-1.7--0.4) | 0.8(-19.3-48.7) | 0.300520038156757 |
| CCL24 | 1.3(-16.4-17.8) | -0.2(-15.9-23.6) | 0.304170137751325 |
| ITGB6 | 0.3(-0.3-0.9) | 0.4(-0.4-11.3) | 0.305876516764549 |
| FCAR | 11.4(-0.3-27.6) | 1.6(-0.5-27.8) | 0.30757756068449 |
| HSD11B1 | 0.7(0.2-16.1) | 0.6(-0.4-14.4) | 0.315733073366085 |
| CRHBP | 0.7(-0.1-14.9) | 0.8(0-16.2) | 0.316729241171609 |
| CLEC4C | 12.6(-0.3-23.5) | 15.4(-0.1-28.1) | 0.322251084476238 |
| TRAF2 | 19.8(-2.2-44.4) | 18.6(-2.7-48.9) | 0.325022559345154 |
| LAIR1 | 0.4(-0.2-13.8) | 0.4(-0.7-12) | 0.33342215782312 |
| CEP164 | 0.2(-0.1-11.7) | 0(-14.1-13.1) | 0.33575904150937 |

Supplementary table 2: Continued

| Protein | Controls | Uveitis | pvalue |
|---|---|---|---|
| CXCL17 | -3.2(-4.1--1.7) | -3.7(-21.6-18.3) | 0.337482325946532 |
| TFF2 | 10.5(-1-25.4) | 2.2(-1.1-25.5) | 0.338402608510915 |
| GZMA | -0.3(-0.6-31) | 1.7(-19.2-16.9) | 0.338895323682067 |
| NRTN | -0.2(-1.1-0.4) | -0.1(-18.1-0.7) | 0.341135749361782 |
| CCL21 | -1.2(-1.6-13.6) | 4.5(-19.7-19.5) | 0.342292953343214 |
| GZMB | -1.2(-1.7-45.3) | 1.7(-39.6-16.9) | 0.342672510026722 |
| EDAR | 29.7(0.9-52.1) | 29.4(0.7-53.5) | 0.351161523260167 |
| CD4 | 0.3(-0.7-9.7) | 0.6(-0.6-14.2) | 0.351384820365002 |
| CCL11 | -0.1(-12.9-0.8) | 0.1(-12.3-0.9) | 0.35406896081721 |
| MEPE | -1(-1.6-10.6) | 2.8(-13.9-14.3) | 0.355835741674836 |
| SPINK4 | 1(0.2-22.4) | 0.8(-0.7-26.8) | 0.357909471591929 |
| CD160 | 12.3(-1-24.6) | 11(-0.7-24.4) | 0.364219291638156 |
| SIGLEC10 | 10(-0.9-23.6) | 2.3(-0.5-19.6) | 0.367027493593631 |
| IKBKG | 29(10-54.7) | 26.8(6-55.1) | 0.36906032795811 |
| DPP10 | 0.1(-0.8-21.6) | 2(-0.4-23.3) | 0.374758150937352 |
| SMOC2 | -0.2(-1-13.9) | 1.4(-9.6-16.9) | 0.375218025091438 |
| SIT1 | -1.8(-2.7--0.2) | -1.6(-19.1-1.1) | 0.378940628470361 |
| LRRN1 | 11.7(0.3-16.6) | 1.1(-0.3-17.3) | 0.379891793314588 |
| ROBO1 | 11.2(-1-26) | 12.8(-0.5-20.8) | 0.384966311734437 |
| KYNU | -2.3(-3.1-17.8) | 0.2(-17.9-16.5) | 0.38747541927483 |
| MAPK9 | 0.7(-0.1-20.4) | 0.9(-0.3-25.1) | 0.389296018238042 |
| JCHAIN | 13.3(-1.1-23.4) | 14.1(-15.2-33.7) | 0.390754690289567 |
| HGF | 16.3(0.3-29.1) | 18(0.5-29.3) | 0.391644443489074 |
| IFNLR1 | 9.1(-1.1-19.8) | 10.6(-0.4-25.5) | 0.393161798853328 |
| ENPP5 | 0.4(-0.6-10) | 0.6(-0.4-12.1) | 0.394628591128091 |
| PRKCQ | 0.2(-0.2-12.2) | 0.3(-0.6-20.3) | 0.402089640553085 |
| SIGLEC1 | 0.3(-1.1-17.6) | 1.3(-0.9-21.5) | 0.402425693784452 |
| SMPDL3A | -0.7(-10.9-13.7) | 0.7(-12-15.4) | 0.405888819820887 |
| RAB6A | 25.9(-1.1-50) | 21.4(-1.7-64.1) | 0.40883283835167 |
| CCL20 | 0.5(-23.7-30.1) | -0.2(-16.2-19.3) | 0.419484147148397 |
| NUDC | 8.5(-1.9-38.1) | 8.2(-1.8-37.4) | 0.422238081638344 |
| LILRB4 | -0.6(-1.7-16.4) | -0.1(-12.5-11.5) | 0.425357831307539 |
| FCRL3 | 0.1(-19.1-16) | 0.3(-17.2-17.7) | 0.428456820958159 |
| IL1RL2 | 0.1(-11.5-13.6) | 0.5(-15.1-11.2) | 0.435548493427224 |
| ADA | 10.1(-0.6-23) | 12.7(-13-21.3) | 0.436091734965378 |
| AMBN | 19.4(-1.9-76.1) | 16(-2-64) | 0.438759847087451 |
| ITGA6 | 0.5(-0.3-18.3) | 0.4(-0.3-13.9) | 0.439125421179183 |
| IL17F | 0(-0.3-18.1) | 0.4(-9.9-25.2) | 0.444247388566596 |
| SCGN | 0(-0.4-13.7) | 0.8(-0.7-18.1) | 0.449960101711722 |
| KRT19 | 0(-1.3-17.6) | 0.7(-1.1-16.4) | 0.44997583551026 |
| CCN2 | 29.6(19.5-34.5) | 30.3(22.4-36.3) | 0.454358639764665 |

Supplementary table 2: Continued

| Protein | Controls | Uveitis | pvalue |
|---|---|---|---|
| WNT9A | -2.9(-22.1--2.1) | -8(-19-1.7) | 0.455200423714291 |
| IL20 | -0.5(-13.3-0.6) | -1.3(-19.5-0.5) | 0.457540773374649 |
| CCL28 | 15.1(0.9-31.5) | 13.3(-1.6-34.1) | 0.459061333205581 |
| IL10RA | 0.2(-0.7-10.9) | 0.3(-0.7-21.6) | 0.463675212886107 |
| IL12RB1 | -0.3(-1-0.2) | 0.2(-16-30.3) | 0.464949434232271 |
| SRPK2 | -0.5(-1.2-35) | 3(-1.3-29.2) | 0.465910050072625 |
| CCL23 | 1.3(0.8-17.8) | 1.6(-0.4-24.6) | 0.467020761739825 |
| FGF19 | -1(-23.9-10.6) | -0.2(-17-11.4) | 0.471410858538393 |
| GOPC | 7.6(-2.5-46) | 3(-2.4-48.3) | 0.47210751571972 |
| TANK | 17.6(-2.4-25.4) | 13.3(-2.3-37.8) | 0.474879602597095 |
| IL10RB | 0.8(0.2-13.5) | 0.8(-0.1-14.9) | 0.476967875489645 |
| IL15RA | 0.3(-0.4-12.7) | 1.1(-0.7-16.4) | 0.487316330294715 |
| ADAM23 | 0(-1-1) | 0.1(-14.8-0.9) | 0.492534116682316 |
| SIRPB1 | 10.2(-0.5-21.8) | 12.5(-0.2-23.6) | 0.494727998453198 |
| IL10 | -51.8(-61.7--17.6) | -52.1(-65.5--27.7) | 0.496439947219278 |
| BTN2A1 | 0.4(-0.5-14.4) | 1.2(-0.4-15.8) | 0.496607634493835 |
| SLAMF1 | 0.7(-0.5-21.7) | 11.2(-0.3-26.6) | 0.497178155599232 |
| LAMA4 | 13.8(-0.3-21.8) | 13.8(-0.2-25.1) | 0.500303697106818 |
| PADI2 | 0.1(-10.6-0.7) | -0.1(-10.8-16.5) | 0.50872599707748 |
| IL1A | 0.4(-12.4-10.4) | 0(-1-22.9) | 0.510078500632364 |
| MILR1 | 10.3(-3-24.4) | 7.5(-8.5-24.1) | 0.513045933589741 |
| IL2 | 0(-0.8-0.9) | 0.3(-9.4-12.3) | 0.513584360744415 |
| MICB_MICA | 9.1(-29.1-18) | 5.9(-48.8-22.4) | 0.51497449511047 |
| IL17A | 0.2(-14.1-1.3) | -0.2(-12-13.9) | 0.519829285554209 |
| CD70 | 10.1(-0.5-18.9) | 1.3(-1.3-20.3) | 0.525162555226524 |
| CXCL12 | 11.6(-3.3-25.8) | 14.7(-2.7-25.4) | 0.525209447572 |
| ICAM4 | 0.6(-0.8-15.2) | 0.5(-9.4-20.7) | 0.529723771393163 |
| COLEC12 | 0.7(0-15.9) | 0.8(-0.1-14.4) | 0.530155616533636 |
| PTX3 | 18(9.2-25.3) | 17.7(-1.4-28.9) | 0.538671111357996 |
| LTBR | 0.6(0-15.3) | 0.6(-0.1-14.5) | 0.565405043894513 |
| LHPP | 13(-1.8-48.6) | 13.6(-2.4-43.8) | 0.565777866814543 |
| CD40LG | 46.8(25.4-49.8) | 44.7(24.6-51.5) | 0.566019385700112 |
| SCGB3A2 | 1.3(-15.7-28.8) | 1(-0.8-25.6) | 0.566790665289044 |
| LTO1 | -0.1(-0.8-0.7) | -0.1(-10.9-0.7) | 0.568605976008299 |
| EGLN1 | 17.9(-5.2-38.5) | 11.2(-5.4-39.5) | 0.571494983529157 |
| CST7 | 20.4(1.2-41.4) | 22.3(0.2-51.8) | 0.581016407020906 |
| IL2RB | 0.1(-0.6-0.7) | 0.1(-1.4-11.1) | 0.587142566154093 |
| MLN | -1.8(-15.8-23.3) | 0.3(-31.7-19.7) | 0.588249757604483 |
| CLEC4D | 26.1(8.9-49.3) | 24(-1-38.4) | 0.597079637445282 |
| PTPN6 | 15.9(-6.1-57.2) | 14.7(-6.5-54.1) | 0.597976212235488 |
| CHRDL1 | -0.3(-1-11.5) | 0.2(-10.1-10.9) | 0.599805630274391 |

Supplementary table 2: Continued

| Protein | Controls | Uveitis | pvalue |
|---|---|---|---|
| PLAUR | 11.6(-0.5-22.3) | 11.3(-1-20.8) | 0.602024574342112 |
| BSG | 1.3(0.4-18) | 1.4(-0.4-15.4) | 0.604993330852544 |
| EPCAM | 0(-0.6-24.2) | 0.1(-19.7-24) | 0.606194642755335 |
| PROK1 | -1.1(-19.5-21.5) | -0.1(-20.2-33.6) | 0.609775621314943 |
| SLAMF7 | 0.8(0.1-21.5) | 0.9(-0.3-30.8) | 0.611662544511206 |
| TNFRSF11A | 1(0.3-19.9) | 10.4(0.3-20) | 0.61338598827534 |
| IL5RA | 0.7(-10.6-12.5) | -0.1(-11.1-19.7) | 0.61653003182152 |
| IL15 | 0.4(-0.1-9.9) | 0.6(-0.3-12.7) | 0.618012044459906 |
| VEGFA | 24.3(10.9-35.5) | 24.7(0.1-46.4) | 0.624237676516179 |
| EIF5A | -0.2(-0.7-0.3) | -0.3(-8.9-1.1) | 0.625103367950942 |
| IL20RA | -0.6(-1.2-13.9) | -0.2(-17.3-33.9) | 0.626011532623788 |
| IL13 | 0.4(0-1.2) | 0.2(-12.4-30.1) | 0.626396048857694 |
| TNFRSF4 | 1.4(-0.1-24) | 11.9(-0.6-22) | 0.628811999354583 |
| RABGAP1L | 11.7(-1.7-39.2) | 8.1(-2-34.8) | 0.628906359553504 |
| IL6 | 12.3(0.8-33.6) | 13.6(-1.3-36.2) | 0.630283370210719 |
| IL16 | 12.2(-0.9-29.7) | 13.6(-1.1-28.3) | 0.630637003422706 |
| CSF1 | 2.1(1.3-22) | 2.1(-0.5-16.8) | 0.635323558358144 |
| HLA-DRA | 1.3(0.6-18.1) | 0.6(-0.4-22.7) | 0.640401700739569 |
| ISM1 | 0.2(-1.1-17.4) | 1.4(-1.2-28) | 0.648339802527412 |
| PTH1R | -0.1(-1.6-0.7) | 0(-10.6-8.6) | 0.659489711520407 |
| ARNT | -11.9(-19.4-0.8) | -13.5(-22-0.2) | 0.664831900219985 |
| CD48 | 0.2(-0.2-13.1) | 1.6(-0.2-17.9) | 0.667826128930755 |
| FCRL6 | 11.5(-0.6-20.7) | 11.2(-1-26.8) | 0.668488855939951 |
| GBP2 | 1.2(-10.4-2.2) | 0.8(-14.2-26) | 0.6686912953104 |
| HCLS1 | 17.9(-4.2-37.9) | 19.8(-5.1-37.8) | 0.671744338474263 |
| MMP1 | 28.3(8.8-40.6) | 30(-8.4-45) | 0.672606346657939 |
| TIMP3 | 68(39-90.1) | 64.2(47.6-103.7) | 0.67265986963019 |
| IL5 | -62.7(-76.1-39.9) | -56.2(-86--9.9) | 0.673215168515826 |
| FOXO1 | 10.8(-3.9-43.9) | 9.9(-3.7-43.7) | 0.67740878756734 |
| CD200R1 | 12.6(-0.1-17.8) | 11.8(-0.3-20.7) | 0.677785321509021 |
| OSM | 28(15.1-53.6) | 28.8(3.4-52) | 0.681267873979829 |
| IL4 | 9.5(-1.1-20.2) | 8.9(-1.9-25) | 0.682148057519357 |
| EPO | 0.6(-0.4-13.7) | 0.1(-13.1-42.1) | 0.682385595818983 |
| LY9 | 0.5(-0.1-14.2) | 1.2(0.2-15.9) | 0.68304928806612 |
| ENAH | 13.1(-1.7-29) | 9.3(-2-27.4) | 0.684606777725423 |
| AMN | -0.1(-19.3-0.8) | -0.2(-15.4-9.9) | 0.690145013253643 |
| ADGRE2 | 0.4(-0.2-17.5) | 1(-0.1-15.6) | 0.693679064113206 |
| GAL | 1.2(-15.3-24.9) | 0.9(-18.6-26.1) | 0.701363142842497 |
| CD22 | 18.8(0.4-34.4) | 18.7(0.5-29) | 0.70172692493061 |
| CLEC4G | 0.1(-0.4-15) | 1.8(-0.4-20) | 0.70787213461309 |
| CSF3 | 12.2(0.2-23.4) | 12.4(0-27.3) | 0.711111839537725 |

Supplementary table 2: Continued

| Protein | Controls | Uveitis | pvalue |
|---|---|---|---|
| FLT3LG | -0.4(-1.2-0.4) | -0.1(-14.3-13) | 0.713952751707993 |
| HLA-E | -0.5(-10.1-10.2) | -0.4(-12.8-1) | 0.716434346257561 |
| FASLG | 9(-1.4-23.2) | 8.8(-0.8-21.3) | 0.7233124462158 |
| PREB | -0.2(-1.1-1.1) | -0.2(-1-11.2) | 0.726923458322354 |
| PRSS8 | -0.2(-0.7-10.7) | 0.1(-0.8-13.4) | 0.729488047857343 |
| GMPR | 16.8(-3.5-44) | 10.9(-3.3-39.3) | 0.731331350003512 |
| ESM1 | 14.4(3.7-20.5) | 12.8(4.2-22.8) | 0.739460712804833 |
| TNFAIP8 | 0.7(0.4-14.8) | 0.4(-0.6-13.6) | 0.740000969801547 |
| AGER | -0.2(-0.4-15.7) | 1.5(-12.2-14.9) | 0.740241439811299 |
| PKLR | 19.5(-2.9-62.2) | 21.8(-7.6-53.6) | 0.740384095142872 |
| KLRB1 | 10.3(-0.4-14.8) | 5.6(-0.3-19.8) | 0.74768481103828 |
| IL1R2 | 0.4(0-0.9) | 0.4(-0.1-0.9) | 0.749885996158964 |
| CD276 | 0.1(-0.7-12.5) | 1.8(-0.8-18.7) | 0.756035096743695 |
| PSPN | -4.2(-39.6-17.7) | -7.6(-44.1-15.8) | 0.761259069240163 |
| FSTL3 | 0.2(-0.3-10.9) | 0.4(-0.6-14.2) | 0.762182415547977 |
| CD6 | 11(-10.5-22) | 10.4(-0.7-25.4) | 0.775321705245148 |
| PRELP | -0.6(-1.8-0.5) | -0.2(-13.3-0.5) | 0.777591754792354 |
| WFIKKN2 | 12.4(-1-32.6) | 12.8(-1.3-30.4) | 0.777687703366549 |
| LY75 | 9.8(-0.8-19.2) | 9.9(-1.1-18.3) | 0.779826160664097 |
| NCR1 | 0.6(-0.6-19.8) | 1(-0.3-17.4) | 0.785474486115314 |
| MMP10 | -0.1(-1.1-23.8) | 1.4(-1.3-27) | 0.789935075068278 |
| CCL7 | 14(-0.6-24) | 12.3(-0.4-43.3) | 0.794241918380344 |
| ANGPT1 | 49.2(40.9-56.8) | 48.8(30.9-54.1) | 0.795236307206967 |
| IL22RA1 | 0.5(-0.6-16.1) | 0.2(-0.7-49.7) | 0.795957784203944 |
| PIK3AP1 | 33.5(12.9-55.2) | 32.7(17.8-61.4) | 0.796687557433992 |
| NELL2 | 19(-1.4-28) | 15.7(-1-25.9) | 0.800917260107163 |
| ITGA11 | -0.4(-12.2-12.7) | 0.3(-19.2-11.9) | 0.801688336284728 |
| TGFA | 22.3(-0.3-46.8) | 24.6(2.6-44.8) | 0.805580994927715 |
| IL3RA | 0.5(-0.6-16.3) | 0.7(0.1-14.5) | 0.806165656891005 |
| CD83 | 10.3(-1-26) | 11.2(-0.5-21.1) | 0.806616967622753 |
| NBN | 8.7(-2.2-33.2) | 6.7(-2.1-29.3) | 0.807631435336808 |
| IL32 | 12.3(-1.2-18.6) | 10(-1.3-22.1) | 0.808367729863938 |
| PAPPA | 0.7(-0.6-21.8) | 0.7(-0.3-17.6) | 0.809005603894149 |
| FGF5 | 0.1(-0.7-14.2) | 0.3(-0.6-24.3) | 0.818375112876028 |
| IL1RN | 0.3(-0.2-18.9) | 1.7(-0.7-24.7) | 0.820266052731225 |
| TGFB1 | 18.1(13.6-23.5) | 18.6(3.5-26.7) | 0.821054283978097 |
| CLEC7A | 0.8(-0.6-15.4) | 0.5(-0.8-19.5) | 0.826886897109885 |
| CCL17 | 48.2(23.6-74.6) | 46.2(29.6-80.3) | 0.830043691800169 |
| CCL22 | 24.9(13.2-36.5) | 25.4(14.8-36.8) | 0.830685241798546 |
| CXCL1 | 41.3(31-53.2) | 43.3(29.8-56.9) | 0.837085127808802 |
| KLRD1 | 0.5(-0.5-17.1) | 0.7(-0.4-18.1) | 0.840914175692723 |

Supplementary table 2: Continued

| Protein | Controls | Uveitis | pvalue |
|---|---|---|---|
| PNLIPRP2 | -1.8(-79.8-17.3) | -0.9(-85.7-22.2) | 0.843932711521783 |
| SLC39A5 | 0.6(-13.4-16) | 0.6(-1-18.4) | 0.845576151179696 |
| AOC1 | -0.2(-0.6-0.3) | -0.4(-12.8-32.5) | 0.860316261300696 |
| CCL13 | 17.9(1.8-28.6) | 17.5(-2.6-29) | 0.861222796479397 |
| NCLN | 0.2(-0.4-0.7) | -0.2(-13.5-26.9) | 0.861442867321617 |
| LGMN | 16.3(0.6-32.7) | 16.2(1.5-30.1) | 0.861772652740414 |
| TLR3 | 0.4(-1-18.1) | 0.9(-12.1-18.5) | 0.863092819730228 |
| LTA | 12.2(-1.2-28.5) | 14.1(-0.6-37.8) | 0.864869545410001 |
| OSCAR | 9.8(-0.5-17.4) | 1.7(-0.4-21.2) | 0.86695181270509 |
| NFATC3 | -0.6(-12.7-1) | -1.1(-17.3-0.8) | 0.871805451607981 |
| LAMP3 | 0(-0.7-18.5) | 0.4(-10.8-19.3) | 0.87261384781928 |
| ITM2A | 11.1(-1.3-17.3) | 10.4(-1.8-31.5) | 0.878187470231777 |
| FXYD5 | 0.2(-0.3-14.6) | 0.3(-0.9-13.9) | 0.880813800434437 |
| IL24 | -0.2(-1.1-0.8) | 0.2(-12.3-12.7) | 0.881629929686243 |
| CTRC | -1.8(-2.5-13.8) | 3.1(-22.9-15.4) | 0.889849227051376 |
| PSIP1 | 11.9(-2.5-53.2) | 12.3(-2.4-38.3) | 0.891183427534975 |
| BCL2L11 | 14.1(-3.8-17.4) | 12.9(-12.7-20.9) | 0.893404680214302 |
| TPP1 | 18.6(11.7-29.1) | 19(3.8-28.5) | 0.894147882992019 |
| IL17RB | 0.2(-0.5-10.9) | 0.4(-0.9-17.4) | 0.895238378279541 |
| TNF | 10.9(-0.7-21.4) | 9.5(-0.2-47.6) | 0.899528645385422 |
| BTN3A2 | 11.7(-0.9-20.4) | 12.9(-0.8-28.8) | 0.913491198316129 |
| NCF2 | 29.8(-7.3-57.6) | 29.1(-6.8-56.5) | 0.913752708529836 |
| SCG3 | 16.3(-1.1-30.1) | 17.4(-1-30.4) | 0.915455456844701 |
| SCGB1A1 | -0.2(-1.3-10.4) | 1(-11.7-14.8) | 0.915650521776439 |
| CLSTN2 | 0.3(-0.3-9) | 0.4(-0.7-12.3) | 0.931624369632655 |
| NFASC | 0.3(-0.2-14.5) | 1.4(-0.2-17.3) | 0.934130833576353 |
| BACH1 | 21.9(6.9-35.4) | 18.9(6.3-47.6) | 0.935069209583791 |
| CCL4 | 24.1(1.4-40.4) | 20.7(11.4-36.6) | 0.935571854938593 |
| CDSN | 0.3(-0.3-13.6) | 0.6(-0.3-17.4) | 0.93829706400392 |
| DNPH1 | 11.7(-2.1-49) | 16.3(-2.4-45.6) | 0.938508976856187 |
| ANGPTL4 | -0.1(-12.5-13.9) | -0.2(-16.6-1.3) | 0.940899023597413 |
| CRLF1 | 0.2(-0.5-9.1) | 0.4(-12.8-11.6) | 0.960959653188432 |
| CLEC4A | 11.1(0.2-16.7) | 10.6(0.1-18.6) | 0.961527624070494 |
| YTHDF3 | 0(-0.6-28.5) | 0.8(-16.7-32.3) | 0.962393145692946 |
| GALNT3 | 9.5(-0.2-17.7) | 1.3(0-21.2) | 0.969099247946806 |
| SELPLG | 0.2(-0.5-0.8) | 0.2(-0.5-0.7) | 0.971674661343724 |
| IL11 | 0.1(-0.6-10.6) | 0.1(-11.7-34.3) | 0.97431501602394 |
| LSP1 | 26.3(14.1-36.4) | 26.7(8-40.5) | 0.979285398722211 |
| NPPC | 20.3(-5.9-36.8) | 22(-4.6-39.2) | 0.988195945647617 |
| ERBB3 | 0.4(0-11) | 0.8(-0.1-15.2) | 0.997814799212795 |

**Supplementary table 3:** Expression data of responders (n=18) and non-responders (n=19). Normalized Protein expression (median [min-max]).

| Protein | Responder | Non-responder | pvalue |
|---|---|---|---|
| BTN2A1 | 1.5(-0.4-13.6) | 0.5(-0.1-1.6) | 0.00145531777234757 |
| OMD | 13.1(-0.8-21.4) | 17.3(2.3-20.2) | 0.00537103232350965 |
| PROK1 | 0.1(-17.9-33.6) | -11.2(-20.2-1.4) | 0.00587608956512308 |
| SIRPB1 | 15.7(1.4-23.6) | 2(-0.1-19.3) | 0.00655792998592823 |
| CKAP4 | 12.2(0.4-26.4) | 15.4(10.3-20.4) | 0.0071750571296908 |
| HLA-E | -0.2(-0.8-1) | -0.5(-1-0.2) | 0.00984158181237215 |
| LRRN1 | 0.9(-0.3-14) | 11.6(0.2-16.2) | 0.0102340936029386 |
| COL9A1 | 74.6(13-95.3) | 84(54.2-100.4) | 0.0125626967436852 |
| OSM | 33.9(3.4-48.3) | 23.9(10.3-39.7) | 0.0126588190501589 |
| YTHDF3 | 0.7(-0.8-17) | 1(-0.7-18.8) | 0.0199872804474353 |
| ICAM4 | 0.6(-0.3-17.1) | 0.2(-9.4-1) | 0.019998271994435 |
| MEGF10 | 12.3(-0.4-20.3) | 15.9(-0.2-22) | 0.0225123459055674 |
| KYNU | -2.2(-17.9-8.4) | 2.8(-6.3-16.5) | 0.0240542630207368 |
| AGER | 1.7(-12.2-9.1) | 1.7(-0.2-14.9) | 0.0253726977632265 |
| SMPDL3A | 0.4(-12-1.8) | 0.7(-1.2-15.4) | 0.0313407301824284 |
| ANGPTL4 | 0.1(-0.9-1.3) | -0.2(-16.6-1.1) | 0.031793415210822 |
| PTX3 | 18.7(12.4-28.9) | 17(-1.4-25.8) | 0.0337338445945058 |
| ISM1 | 0.8(0.2-20.1) | 11.4(-0.3-16.3) | 0.0337584889311562 |
| WFIKKN2 | 5.8(-0.7-28.4) | 17.3(-0.5-21.4) | 0.0357276285399068 |
| CRIM1 | 1.4(0.3-15.8) | 0.5(0.1-1.6) | 0.0360609083918354 |
| SMOC2 | 1.1(-9.4-11.4) | 9.5(-9.6-15) | 0.0370629472774663 |
| FGF2 | 1(-0.5-23.6) | 0.4(-0.6-12.7) | 0.0371819797262365 |
| CXCL17 | -5.1(-17-1) | -3.3(-13.1-18.3) | 0.0373908778920246 |
| CDON | 10.5(-0.8-15.9) | 13.3(-0.4-21.4) | 0.040580795277836 |
| SLAMF7 | 11.7(-0.2-30.8) | 0.8(0.2-15.4) | 0.0457733938638779 |
| ITGA11 | 0.2(-19.2-1.3) | 0.4(-0.7-11.9) | 0.0494955893377805 |
| IL17RB | 0.6(-0.8-17.4) | 0.3(-0.7-0.9) | 0.0540380596674024 |
| TGFA | 25(14.9-44.8) | 20.8(10.9-36.2) | 0.0561952798580275 |
| IFNLR1 | 12.1(1.3-20.8) | 10.6(0-15) | 0.0597800353124845 |
| ADAM23 | -0.2(-14.8-0.8) | 0.1(-0.8-0.7) | 0.0605207140209581 |
| GZMA | 1.4(-19.2-8.5) | 1.8(-0.6-13.7) | 0.0647030227588124 |
| PKLR | 20.9(-7.6-38.4) | 24.3(6.1-53.6) | 0.0649469094632809 |
| IL20 | -10.3(-16.4-0.5) | -1.3(-14-0.2) | 0.0682597291288868 |
| ROBO1 | 12.3(-0.4-18.1) | 14.4(1.8-20.8) | 0.0685302500268365 |
| TNFRSF13B | 0.6(-0.1-13.4) | 0.5(0.1-1.3) | 0.0702813961980298 |
| DGKZ | 0.4(-0.3-0.9) | 0.6(-0.2-0.9) | 0.0726499458075999 |
| LHPP | 4.6(-1.9-27.5) | 14.6(4.3-43.8) | 0.0733821057383125 |
| FXYD5 | 0.4(-0.3-0.8) | 0.5(-0.7-13.6) | 0.0746429677154645 |
| CLEC4C | 16(1.1-28.1) | 12.2(0.5-19.4) | 0.076159774504284 |
| CSF3 | 13.3(0.7-26.5) | 1.1(0.2-27.3) | 0.0783472114735366 |
| B4GALT1 | 0.4(0.1-0.8) | 0.5(-0.2-10.9) | 0.0804591046436417 |

Supplementary table 3: Continued

| Protein | Responder | Non-responder | pvalue |
|---|---|---|---|
| SLAMF1 | 11.9(0.7-19) | 0.9(-0.3-18.5) | 0.0885067118713848 |
| IL16 | 14.6(2.4-24.7) | 11.1(-0.7-28.3) | 0.0982678062293879 |
| LGMN | 16.8(13-30.1) | 15.6(11.6-22.1) | 0.103009644927995 |
| WAS | 1.5(-0.7-8.4) | 1.8(-1.1-28.9) | 0.103288187254629 |
| MMP1 | 30.7(12.8-45) | 26.5(-8.4-35.1) | 0.105350862455321 |
| PPP1R9B | 25.8(16.3-44.3) | 21(-4.6-53.3) | 0.106757566319841 |
| ADA | 12.7(-13-21.3) | 13.3(-0.4-19.4) | 0.10687432363171 |
| TNFSF11 | 17.8(1.2-28.1) | 13.7(0.1-25.6) | 0.112009465381399 |
| IL33 | 0.3(-0.8-12.5) | 0.4(-0.5-11.9) | 0.112234449199677 |
| HGF | 18.3(11.2-29.3) | 16(1.3-22.7) | 0.113969493497148 |
| CRLF1 | 0.7(-0.1-9.9) | 0.4(-12.8-1.5) | 0.115222033689157 |
| CTSO | 1.4(-0.1-14.8) | 1.7(0-16.8) | 0.116358377324937 |
| MEPE | 2.8(-10.6-9) | 3.2(-1.1-9.9) | 0.117064932996221 |
| PNLIPRP2 | 1.4(-80.4-22.2) | -1.3(-82.2-2.8) | 0.128481454738725 |
| LY6D | 0.6(0-13.2) | 0.5(-0.6-1.4) | 0.128515787336145 |
| CDSN | 1.2(-0.2-17.4) | 0.7(-0.3-12.9) | 0.128762030963528 |
| NPPC | 22(5.9-36.6) | 26.9(6.5-39.2) | 0.129485250337932 |
| PRELP | -0.2(-1.1-0.3) | -0.3(-10.6-0.2) | 0.129752046250039 |
| NELL2 | 13.9(2.3-25.9) | 19.1(3.1-24) | 0.13188133243633 |
| IFNGR1 | 0.6(0.2-1.7) | 0.5(0-1.6) | 0.132126840876658 |
| IL32 | 5.8(-1.1-20.4) | 11.6(-1.3-19.2) | 0.133379057796491 |
| OSCAR | 11.5(0.2-18.2) | 1.7(-0.1-15.2) | 0.137950803104847 |
| NCLN | -0.2(-0.7-15.8) | -0.1(-0.8-0.6) | 0.140669881156106 |
| CD276 | 1.3(-0.4-14.3) | 1.8(-0.4-17.2) | 0.145466151774006 |
| LY75 | 5.9(-0.9-17.9) | 11.5(2.1-15.8) | 0.147251383178372 |
| ITGB6 | 0.5(-0.4-0.9) | 0.5(-0.3-11.2) | 0.149428686846465 |
| SCGN | 0.9(-0.1-1.7) | 0.7(-0.5-18.1) | 0.149612614119243 |
| IL5RA | 0.2(-9.9-19.7) | -0.4(-10.4-0.7) | 0.151642958863692 |
| LGALS4 | 2.1(-14.4-3.5) | 2.3(-2.4-12.4) | 0.159857472145885 |
| PTH1R | 0(-8.6-0.8) | 0.2(-0.8-0.7) | 0.160811385236063 |
| LAMA4 | 12.5(0-18.5) | 15.4(-0.1-20.5) | 0.163390174057654 |
| REG4 | 0.6(-0.7-13.8) | 0.4(-0.7-13.5) | 0.168735518431858 |
| LAIR1 | 0.7(-0.3-12) | 0.4(-0.7-11) | 0.175583371417034 |
| TNFRSF13C | 1.8(-0.1-22.9) | 12.7(0-20.1) | 0.182940904817392 |
| PRSS8 | 0.2(-0.6-10.8) | 0(-0.6-0.9) | 0.185329539282474 |
| FLT3LG | 0(-14.3-0.7) | -0.1(-1.2-13) | 0.18697754463604 |
| ADGRE2 | 0.9(0.1-13.8) | 1.3(0-14.1) | 0.189343244651726 |
| IL6 | 16.8(-1.3-36.2) | 10.4(-0.5-31.4) | 0.189497230157201 |
| VEGFD | 0.3(-11.4-1.1) | 0.4(-0.8-12.7) | 0.192361655356532 |
| CCL11 | 0.2(-12.3-0.9) | 0.2(-0.8-0.6) | 0.193578071463369 |
| ACTN4 | -0.1(-0.5-13.8) | 0.1(-0.2-0.6) | 0.194373579246969 |

Supplementary table 3: Continued

| Protein | Responder | Non-responder | pvalue |
|---|---|---|---|
| PCDH1 | 0.5(-0.2-1.3) | 0.6(0.3-8.7) | 0.20128392919041 |
| MICB_MICA | 9.6(-28-18) | 3.1(-40.7-22.4) | 0.205205598991992 |
| ITGA6 | 0.4(0-0.9) | 0.3(-0.3-13.6) | 0.20919990349269 |
| ANGPT1 | 51.1(30.9-54.1) | 47.9(41.5-51.4) | 0.212567187259242 |
| CD22 | 18.8(1-27.1) | 20.5(12.7-29) | 0.212690078677036 |
| ALDH3A1 | 2.3(-1.3-33.8) | 1.3(-1.1-19.3) | 0.215099670356195 |
| ANXA11 | 18.2(-2.1-37.5) | 7.9(-2.1-38.3) | 0.219140691271664 |
| LAP3 | 0.2(-0.6-16.4) | 0.3(-0.7-16.2) | 0.220741064220061 |
| JUN | 1.1(-1.6-48.7) | -0.3(-1.5-1.6) | 0.221131893370295 |
| SCG3 | 16.5(2.3-28.7) | 22.1(3-28.8) | 0.22248676729305 |
| LIFR | 6(0.6-14.8) | 10.7(0.3-14.6) | 0.224556259464486 |
| CD40 | 12.7(1-23.3) | 9.7(0.5-21.5) | 0.23215805954369 |
| CTRC | 3.4(-11-14.5) | 2.9(-12.5-10.8) | 0.232183375238335 |
| CCL23 | 10.2(-0.2-24.6) | 1.4(-0.4-19.9) | 0.2395276516335 |
| IL17A | -0.2(-1-13.9) | -0.2(-10.7-1.2) | 0.242103500248226 |
| MATN2 | 13(-1.1-19.5) | 13.5(-0.8-20.5) | 0.249254710627956 |
| MZB1 | 0.9(0-25) | 0.4(-0.8-14) | 0.25247770352544 |
| EDAR | 30.5(14.4-49.6) | 26.9(0.7-49.6) | 0.253552933723484 |
| FST | 0.7(-0.9-15.9) | -0.1(-14.2-15.7) | 0.254853074719224 |
| GZMB | 1.7(-39.6-3.1) | 1.8(-12.8-12.3) | 0.258821193216932 |
| PON3 | 0.5(-0.2-1) | 0.3(-0.5-0.9) | 0.261437689070336 |
| LAT | 27.6(17.9-37.2) | 27.4(23.7-37.3) | 0.262532208415467 |
| LY9 | 1.1(0.2-13.6) | 9.8(0.2-15) | 0.263408944960889 |
| CD200R1 | 12(0-20.7) | 12.3(0-18.6) | 0.269133726211198 |
| CD84 | 14.7(4.3-17.8) | 14.2(4.6-16.7) | 0.269173324936588 |
| FASLG | 2.4(-0.7-15.8) | 9.8(-0.8-14.9) | 0.269185275421547 |
| TLR3 | 0.7(-12.1-15.7) | 1.1(-0.5-16) | 0.272896679813442 |
| CD244 | 2.2(0-16.3) | 10.2(-0.1-15.8) | 0.273287930017158 |
| DECR1 | 4.2(-8.4-10.2) | 4.6(-2.1-35.8) | 0.276621865930459 |
| EIF5A | -0.3(-1.2-0.5) | -0.2(-0.7-0.5) | 0.27679243358062 |
| NCF2 | 27(11.6-47.4) | 25.6(8.1-47.2) | 0.277349865982893 |
| LTO1 | -0.1(-0.5-0.6) | -0.1(-10.9-0.6) | 0.287912422555697 |
| IL2 | 0.4(-0.2-0.8) | 0.2(-1-12.3) | 0.289126638611894 |
| IL18 | 1(-0.6-2.1) | 0.3(-1.1-20.6) | 0.29474393935699 |
| SLC39A5 | 0.6(-1-14.3) | 0.6(-0.4-10.8) | 0.295552544245381 |
| PSMG3 | 8.5(6.9-28.8) | 18.5(-3.8-42.5) | 0.300567582772259 |
| CLEC7A | 0.6(-0.4-13) | 0.5(-0.8-11.1) | 0.303441920211581 |
| NME3 | 1.3(-0.1-15.9) | 1.9(0.1-9.5) | 0.303857607452588 |
| PRDX3 | 0.3(-0.5-14) | 0.3(-0.2-21.7) | 0.303963606751506 |
| AGRN | 1.3(-0.2-13.9) | 0.8(-0.3-14.5) | 0.307156722572391 |
| HPCAL1 | 14.7(1.4-30.9) | 18.2(0.1-36.4) | 0.30896741552552 |

Supplementary table 3: Continued

| Protein | Responder | Non-responder | pvalue |
|---|---|---|---|
| IL24 | 0.2(-0.6-1) | 0.4(-10.5-12.7) | 0.310076396279573 |
| CCL24 | 0.7(-2.6-23.6) | -0.8(-10.7-17.1) | 0.311029708001545 |
| SIGLEC10 | 5.6(-0.5-19.6) | 2.3(-0.4-15.7) | 0.311901620095135 |
| TGFB1 | 18.7(13.2-24.3) | 17.6(3.5-26.7) | 0.314117250753797 |
| TNFRSF14 | 16.7(9.9-21.5) | 14.6(11-21.6) | 0.317992738600688 |
| IL20RA | 0.1(-1.1-1.5) | -0.1(-11.5-1.6) | 0.320658337617763 |
| DPP10 | 2(-0.1-17.7) | 9(0-15.4) | 0.323650172733248 |
| GOPC | 3.1(-1.7-35.4) | 3.3(-1.9-41.8) | 0.326399072851556 |
| TANK | 15.8(3.7-26.3) | 12.4(-2.1-37.8) | 0.327987713901515 |
| PLAUR | 12.1(2-16.4) | 9.7(-0.6-18.8) | 0.328178032037777 |
| HCLS1 | 21.6(6.1-29.6) | 15.3(5.4-37.8) | 0.331750308995939 |
| IL17D | 0.3(-0.4-20.6) | 0.3(-0.3-1.2) | 0.334384107168689 |
| CD6 | 10.2(-0.7-19.6) | 12.4(0-25.4) | 0.335714799560811 |
| CD70 | 1.2(-0.5-16.1) | 0.5(-1.3-17.5) | 0.336080248025444 |
| SIT1 | -1.3(-19.1-0.5) | -1.5(-9.6-0.5) | 0.338838178413438 |
| LGALS9 | 0.7(0.4-13.6) | 0.6(0.2-13.8) | 0.348686938946943 |
| EGF | 62(47-75.2) | 59.5(41.3-70.7) | 0.349556324676951 |
| BID | 18.2(-1.6-49.6) | 15.7(-1.3-38.1) | 0.350181650953369 |
| IL10RA | 0.3(-0.1-11.2) | 0.4(-0.5-21.6) | 0.35198666952495 |
| LAMP3 | 0.6(-10.8-18.1) | 0.4(-0.5-14.7) | 0.355077595256849 |
| MMP10 | 1.3(-1-18.7) | 1(-0.4-22.1) | 0.362505571702733 |
| CNTNAP2 | 17.9(-2.2-34.6) | 20.4(12.5-28.8) | 0.366662276766863 |
| DAPP1 | 0.7(-16.1-2.4) | 0(-14-18.7) | 0.366949126419952 |
| IL1RN | 6.1(-0.1-20.4) | 0.9(-0.5-24.7) | 0.367068215574393 |
| CST7 | 22.7(1.2-47.7) | 20.4(1.2-39.6) | 0.371526455089935 |
| CCL4 | 23.8(11.4-29) | 20.4(11.5-32.4) | 0.371843857261377 |
| DBNL | 40.7(25.5-70.9) | 31.1(18.8-64.1) | 0.373689244642468 |
| TNFRSF11A | 10.8(0.4-18.3) | 10.4(0.3-20) | 0.375476285257153 |
| CLEC4D | 25.7(-0.4-36.2) | 21.7(2.8-37.7) | 0.379911610727714 |
| MERTK | 0.7(-0.3-1.8) | 0.8(0.1-9.9) | 0.37999260002037 |
| CCL26 | 29.8(22.7-53.7) | 29.1(1.7-39.1) | 0.400338580914995 |
| ESM1 | 9.2(8.4-21.9) | 14.5(8.6-19.4) | 0.402559582308354 |
| CSF1 | 2.1(-0.4-12.8) | 2.1(-0.4-16.8) | 0.402930996419729 |
| IL2RB | 0.1(-1.4-1.4) | -0.1(-0.8-0.7) | 0.405319312074252 |
| DNPH1 | 17.7(-1.8-31.3) | 17.2(-1.9-43.8) | 0.405910213463703 |
| AOC1 | -0.4(-1.1-0.2) | -0.3(-8.8-32.5) | 0.416845729652882 |
| AMBN | 14.7(-0.6-62.2) | 24.8(-0.4-62.8) | 0.418493041541968 |
| CXCL8 | 16.3(1.6-65.9) | 16.5(-0.4-25.6) | 0.418586945170601 |
| CKMT1A_CKMT1B | 0.6(-41.8-15.8) | -1.1(-15-1.9) | 0.42027828725435 |
| LTBR | 0.6(0-11.8) | 0.7(0.1-11.1) | 0.4209752275722 |
| SPINT2 | 15.2(6.1-24.3) | 16.2(6-24.5) | 0.424752478334142 |

Supplementary table 3: Continued

| Protein | Responder | Non-responder | pvalue |
|---|---|---|---|
| AGRP | 2.1(-1.3-16.7) | 2.5(-9.8-16.6) | 0.425458817286109 |
| FGF5 | 0.3(0-1.1) | 0.3(-0.6-12.5) | 0.429495621774053 |
| LSP1 | 27.5(15.4-34.7) | 23.7(14.4-40.5) | 0.432371027077516 |
| IL4R | 5.7(0.1-16.9) | 1.7(0-14) | 0.435393939648678 |
| CXCL10 | 13.4(-1.1-24.8) | 13(-0.8-27.1) | 0.437331402000068 |
| SAMD9L | 28.2(18.6-54.1) | 22.7(14.1-49.4) | 0.440032360031103 |
| CD200 | 1.5(-0.1-17.7) | 1.4(0-12.9) | 0.443335786122828 |
| ENAH | 6.5(-1.7-23.6) | 11.9(-1.7-21.7) | 0.444340191036089 |
| HEXIM1 | 8.2(6.3-33.5) | 12.6(6.3-43.2) | 0.448232392289626 |
| LTA | 14(-0.4-21.6) | 14.6(-0.6-25.1) | 0.452523910071612 |
| FGF19 | -0.4(-13.4-11) | -0.1(-17-11.4) | 0.453183887711475 |
| RAB6A | 23.9(-1-58.8) | 20.5(2.9-50.7) | 0.454579359758784 |
| CCL7 | 14.4(-0.1-43.3) | 12.4(-0.1-29.5) | 0.456503132500575 |
| TFF2 | 2.3(-0.4-21.6) | 2.2(-0.7-15.7) | 0.458907329354833 |
| CCL20 | -0.2(-15.8-19.3) | 0.2(-16.2-10.9) | 0.461131929553861 |
| SULT2A1 | 0(-1.2-12.1) | 0.1(-0.7-10.8) | 0.463769480773978 |
| NTF3 | 0.3(-13.2-11.7) | 0.5(-0.2-11.4) | 0.471802508775457 |
| FCAR | 7.2(0-19.8) | 1.6(-0.5-17.5) | 0.472804803602559 |
| MANF | 46.6(32.8-77.2) | 45.3(30.3-77.4) | 0.473302197682597 |
| PDLIM7 | 39.8(14.7-79.6) | 35.1(12.3-74.3) | 0.473643121564267 |
| TNF | 6.8(0.2-16.7) | 10.8(0.1-17.8) | 0.476911229578607 |
| MILR1 | 6.2(-2.4-22.8) | 13(-2.5-19.9) | 0.476925080070253 |
| GAL | -0.3(-11.8-24.7) | 0.9(-18.6-15.2) | 0.479464610434761 |
| CLEC4A | 10.7(0.1-17.8) | 10.4(0.2-15.6) | 0.479909790701709 |
| RGS8 | -0.2(-1.1-17.8) | -0.2(-0.6-22.7) | 0.480094619866416 |
| FKBP1B | 15.7(-2.5-57.8) | 13.5(-2.2-44.1) | 0.480528796962779 |
| CRELD2 | 13.4(0.4-26.3) | 13.4(0.1-19.7) | 0.480753961351084 |
| CD79B | 15.6(3.1-31.3) | 16.9(3.6-25.2) | 0.4862064834735 |
| TREM2 | 0.1(-12.3-11.4) | 0.1(-1-13.2) | 0.487299124521053 |
| GBP2 | -0.7(-2.1-11.3) | 1.1(-12.8-26) | 0.489415552050151 |
| CCL13 | 19.9(-2.5-29) | 18.2(-1.6-26.7) | 0.489845090714889 |
| EPHA1 | 0(-9.9-0.7) | -0.1(-1-0.5) | 0.490470763985179 |
| SCGB1A1 | 1.3(-0.5-14.8) | 0.8(-10.4-13.4) | 0.500336829576293 |
| TNFRSF11B | 0.4(-0.3-1.4) | 0.2(-0.1-8.1) | 0.52418169810879 |
| AMN | -0.2(-15.4-0.4) | -0.1(-14.6-0.8) | 0.527306595072039 |
| RABGAP1L | 4.2(-1.6-34) | 8.6(-1.2-30.8) | 0.53189437546658 |
| CXCL14 | -1.4(-15.1-25.6) | -1.7(-14-1.9) | 0.533131036285317 |
| TPSAB1 | 0.6(-14.7-11.5) | 0.3(-2.1-11.7) | 0.535173889815223 |
| BANK1 | 29.3(12.1-61.7) | 30.7(13.1-67.5) | 0.540744839220387 |
| CCN2 | 30.4(24.1-35.4) | 30.5(22.4-36.3) | 0.543766983660029 |
| SCGB3A2 | 0.5(-0.3-18.7) | 1.1(-0.8-22.6) | 0.545733240861531 |

Supplementary table 3: Continued

| Protein | Responder | Non-responder | pvalue |
|---|---|---|---|
| FCRL6 | 12.6(-0.3-26) | 10.4(-0.7-26.8) | 0.554466688017739 |
| IL1RL2 | 0.8(0.1-8.5) | 0.7(-0.3-11.2) | 0.554905673989437 |
| SH2D1A | 2.6(-1.2-21.1) | 2.4(-1-15.6) | 0.565578798529326 |
| PRKCQ | 0.5(-0.4-1.7) | 0.3(-0.4-8.4) | 0.573629292934289 |
| IL1R2 | 0.4(0.2-0.9) | 0.4(0.1-0.7) | 0.57376756500758 |
| SPINK4 | 1(-0.2-26.8) | 0.3(-0.2-26.1) | 0.5746102211453 |
| ERBB3 | 0.9(0.5-15.2) | 1(0.3-15.2) | 0.579976303271528 |
| CEP164 | 0(-1.1-13.1) | 0.2(-14.1-9.7) | 0.583701409303214 |
| IL11 | 0(-11.7-14.1) | 0(-10-0.7) | 0.587357361748675 |
| NFATC1 | 20.8(16.6-46.4) | 19.7(5.8-52.6) | 0.588472363801341 |
| ARNT | -11.6(-22--0.5) | -13.6(-19.4--0.1) | 0.595342999771407 |
| SIGLEC1 | 1.5(0.1-13.7) | 1.2(-0.9-16.9) | 0.595662884210698 |
| GMPR | 9.6(5.5-38.4) | 17.6(7.2-39.3) | 0.595912886963402 |
| WNT9A | -8.8(-16.4-1.7) | -8.1(-15.6-1.7) | 0.607750847658994 |
| NBN | 8.5(-1.5-23.2) | 4.4(-1.8-28.1) | 0.609345390757327 |
| TBC1D5 | 23(15.8-58.9) | 18.9(13-48.8) | 0.610308752236288 |
| PTPRM | 10.8(0.8-16.4) | 10.8(0.6-14.6) | 0.618156637519991 |
| IRAK1 | 19.5(12.8-33.3) | 16.9(4-37.5) | 0.618193274116991 |
| RAB37 | 11.2(-1.7-42.2) | 14.9(-1.5-28.7) | 0.619429252416392 |
| IL1A | 0.1(-1-18.1) | 0.1(-0.7-22.9) | 0.619510463939451 |
| IL17F | 0.5(-0.4-25.2) | 0.5(-0.6-20.1) | 0.623954019583544 |
| LILRB4 | -0.1(-12.5-11.5) | -0.1(-11.5-0.7) | 0.625653897517984 |
| FIS1 | 5.7(-0.6-39.5) | 0.4(-0.7-31.2) | 0.62876768059873 |
| DNAJA2 | 41(28.8-71.1) | 38.3(24.1-67.8) | 0.636249124428104 |
| IL5 | -55.9(-73.2--22.1) | -55.3(-74.3--26.2) | 0.638086070787709 |
| EPO | -0.1(-0.9-23.5) | 0.3(-10.6-24.7) | 0.642510068842724 |
| CD4 | 0.7(-0.6-13.2) | 0.7(0.1-14.2) | 0.644919334855964 |
| GLOD4 | 12.6(-2-25.5) | 13.9(-2-33.1) | 0.644946636050611 |
| BSG | 1.2(-0.2-14) | 1.5(0.2-14.4) | 0.65015397811667 |
| NFASC | 1.4(0.1-17.3) | 1.4(-0.1-12.3) | 0.654980036709441 |
| TPT1 | -0.1(-11.2-19.6) | 0(-10.9-12.8) | 0.656595479828258 |
| CCL22 | 27(16.2-33) | 24(15.6-36.8) | 0.657145752949107 |
| CCL28 | 15.1(-1.2-27.3) | 12.6(-1.6-31) | 0.658336664812476 |
| ENPP7 | 0(-23.1-9.3) | -0.2(-23.3-12.4) | 0.659841004084723 |
| PGF | 0.6(-0.2-14) | 0.7(0.3-13.9) | 0.660535559319687 |
| DAG1 | 19.7(2.4-30.4) | 17.1(2.3-26.6) | 0.673163857792048 |
| BACH1 | 19.9(7.1-37.5) | 17.3(6.9-42.8) | 0.67452864946124 |
| SPON1 | 1.4(-0.4-11.2) | 0.9(-0.1-17.8) | 0.679228278154883 |
| CLEC4G | 1.9(0.1-20) | 1.7(-0.2-17.1) | 0.681830217019127 |
| KRT19 | 1.1(-0.4-8.5) | 0.3(-1.1-16.4) | 0.682060818679351 |
| NT5C3A | 22(6.1-62.1) | 20.7(6-62.2) | 0.689172038530612 |

Supplementary table 3: Continued

| Protein | Responder | Non-responder | pvalue |
|---|---|---|---|
| KLRD1 | 0.7(-0.1-14.5) | 1(-0.2-15.8) | 0.690361396857927 |
| NUB1 | 14.3(4-35.7) | 14(4.4-41.6) | 0.694605394681103 |
| EPCAM | 0.1(-19.7-21.9) | 0.2(-1.1-24) | 0.695676449897625 |
| NCR1 | 1(0.3-17.4) | 1.1(0-16.5) | 0.699942423371019 |
| CD83 | 12.5(1.4-20.8) | 12.6(-0.3-21) | 0.701648661003402 |
| COLEC12 | 0.9(-0.1-12.3) | 0.8(-0.1-13.5) | 0.702845315991833 |
| CLSTN2 | 0.1(-0.7-9.5) | 0.5(-0.1-1.2) | 0.708914720781111 |
| FCRL3 | 0.4(-13.8-17.7) | 0.3(-1-13.4) | 0.709635331356212 |
| TRAF2 | 18.6(-2.7-28.6) | 20.4(4.2-46.8) | 0.710887411355003 |
| IL10RB | 0.7(-0.1-11.3) | 0.7(0-11.7) | 0.715732336893194 |
| IDS | 0.2(-0.3-0.4) | 0.2(0-0.3) | 0.716144481417394 |
| IL4 | 2.5(-0.6-25) | 2.5(-0.9-16.5) | 0.719889449247649 |
| VEGFA | 26.7(1.8-35.1) | 22.4(0.1-43.8) | 0.720500313899248 |
| FSTL3 | 0.5(0-14.2) | 0.3(-0.6-13.2) | 0.721110302085291 |
| GALNT3 | 5.4(0.3-21.2) | 13(0.1-21.1) | 0.728212847608644 |
| IL12RB1 | 0.3(-0.7-1.9) | 0.2(-0.8-1.7) | 0.729524990136519 |
| CTSC | 11.3(0.3-30.9) | 13.3(-0.2-20) | 0.730382019566266 |
| PLA2G4A | 4.9(-1.8-26.3) | 4.8(-1.8-36.1) | 0.731521407739367 |
| HSPA1A | 3.8(-1.5-27.5) | 3.4(-1.6-37.3) | 0.735191052932307 |
| JCHAIN | 13(-0.4-24.2) | 15(-15.2-33.7) | 0.739028085047216 |
| IL3RA | 0.7(0.3-11.6) | 0.7(0.3-13.4) | 0.739377629542943 |
| CEACAM21 | 0(-12.1-30.3) | 0.7(-10.2-22.9) | 0.741448240081727 |
| PADI2 | 0(-10.8-12.2) | -0.1(-1.1-0.3) | 0.742187061369115 |
| MVK | 7.3(-12.5-22.4) | 7.3(-2.6-16.5) | 0.743331905453546 |
| SELPLG | 0.2(-0.3-0.5) | 0.2(-0.5-0.7) | 0.745807289737754 |
| FABP9 | 10.9(0.5-23.8) | 11.5(-0.7-18) | 0.746146338367553 |
| IKBKG | 27.7(18.1-49.8) | 24.5(9.1-55.1) | 0.747533671033696 |
| PNPT1 | 5.3(-12.3-46.2) | 12(-9.7-43.3) | 0.750580349108695 |
| CD40LG | 44.9(24.6-51.2) | 44.8(25.8-50.3) | 0.753071973039558 |
| IL1B | 0.6(-0.1-24.5) | 0.4(-0.5-11.2) | 0.766017882528802 |
| CD160 | 11.8(-0.1-21.5) | 12.5(-0.6-17.5) | 0.766769617310998 |
| CCL25 | 0.5(-0.4-17.2) | 0.5(-0.2-12.5) | 0.768063635135141 |
| SKAP2 | 54.2(17.6-83.8) | 51.8(23.7-74.6) | 0.768444047165684 |
| CLIP2 | 22.9(3.7-44) | 20.4(13.1-61.4) | 0.769063926268548 |
| SRPK2 | 3.1(-1-18.2) | 3(-1.2-26.8) | 0.769978163598603 |
| PTPN6 | 20(8.6-39.6) | 11.5(6.3-54.1) | 0.770076745563759 |
| MAP2K6 | 33.4(23.2-62.3) | 33.8(18.9-58.5) | 0.770464370284415 |
| DNER | 1.4(-0.4-15.7) | 1.7(-0.5-12.5) | 0.772619537222702 |
| MPIG6B | 46.1(21.3-56.1) | 44.8(29-57.3) | 0.774520315661512 |
| CASP2 | 16.3(3.8-53.2) | 13.7(-1.7-40.3) | 0.779959199480175 |
| PAPPA | 0.8(-0.3-12.5) | 0.8(0-12.3) | 0.784138731963231 |

Supplementary table 3: Continued

| Protein | Responder | Non-responder | pvalue |
|---|---|---|---|
| PRDX5 | 25.7(9.4-45.1) | 23.6(12.3-42.8) | 0.794201339823273 |
| CXCL9 | 0.5(-0.8-24.5) | 0.4(-10-28.4) | 0.796450034652841 |
| IL12B | 14.5(-10.5-25.3) | 15.1(0.7-25) | 0.802145680807354 |
| KLRB1 | 1.8(-0.3-19) | 1.9(-0.1-17.9) | 0.804022113574244 |
| MGLL | 21.7(3.2-38.8) | 18.6(-1.4-42.3) | 0.805129529373724 |
| BCL2L11 | 11.8(-3.4-20) | 11.9(-3.7-17.9) | 0.809660492664547 |
| NUDC | 10.2(2.6-22.5) | 9.8(-1.6-34) | 0.81063998051373 |
| CXCL6 | 36(31.8-49.3) | 39.2(25.2-53.9) | 0.814443616437224 |
| EGLN1 | 10.7(8.4-35) | 11.5(8-37.5) | 0.815310869123559 |
| IL22RA1 | 0.4(-0.4-49.7) | 0.2(-0.7-22.2) | 0.818357407310329 |
| TNFRSF4 | 12.1(-0.6-20.9) | 12.6(0.3-19.2) | 0.819643786288546 |
| NCK2 | 1.6(-0.5-23.7) | 1.4(-0.7-38.5) | 0.825878930357735 |
| IL17C | -3.2(-16.7-10.5) | -3.2(-13.4-3.6) | 0.82717728251897 |
| CRHBP | 0.9(0.2-15.8) | 0.8(0-13.1) | 0.827432997705092 |
| IL13 | 0.1(-0.6-13.1) | 0.3(-12.4-30.1) | 0.82914604992976 |
| CXCL1 | 43.5(33-53) | 43.2(29.8-56.9) | 0.829708241069608 |
| CRKL | 35.4(22.1-67.8) | 33(5.8-67.1) | 0.831644101358158 |
| PREB | -0.1(-0.6-0.5) | -0.1(-0.6-1.5) | 0.836580557215971 |
| TRIM5 | 10.3(3.9-44.9) | 13.5(-2.6-31.5) | 0.838870896322728 |
| ATP5IF1 | 26.2(1.3-47.4) | 24.1(1.4-49.2) | 0.839449881684244 |
| F2R | -15.8(-25.4--6.5) | -15.5(-29.3--4.2) | 0.839620085455909 |
| PSPN | -4.1(-33.2-15.8) | -9.3(-44.1-3.2) | 0.840535812949686 |
| TNFSF10 | 2.9(-0.3-18.8) | 3(-0.4-14.9) | 0.847692382913093 |
| CCL21 | 4.6(-19.7-12.5) | 4.3(-1.5-11.7) | 0.84926866669499 |
| C1QA | 0.2(-0.3-0.6) | 0.2(-0.4-0.5) | 0.850600142083284 |
| VASH1 | 0.1(-0.6-1.2) | 0.1(-0.5-1.4) | 0.853116746798292 |
| PARP1 | 0.3(-0.4-17.5) | 0.2(-0.3-36.7) | 0.854925089830114 |
| NFATC3 | -1.2(-12.5--0.5) | -0.9(-12.1-0.8) | 0.856293778544184 |
| HSD11B1 | 0.5(-0.4-13.1) | 0.5(-0.1-9) | 0.857756964987256 |
| ANGPTL2 | 2.4(-10.3-12.4) | 2.1(-1.5-10.9) | 0.858796325745866 |
| ENPP5 | 0.8(-0.1-12.1) | 0.7(-0.3-11.9) | 0.868237484075601 |
| MYO9B | 1.8(-0.5-26.9) | 0.9(-1.5-20.4) | 0.869006085710811 |
| BCR | 8.6(5.1-34.9) | 7.7(-3.1-43.4) | 0.870016485147252 |
| PIK3AP1 | 33.3(23.2-45.2) | 35.6(17.8-61.4) | 0.872352119895806 |
| AXIN1 | 20(7.8-44.5) | 18.4(7.8-53.5) | 0.886141706581545 |
| BTN3A2 | 13.7(2.3-17.4) | 12.8(-0.6-28.8) | 0.886145155590523 |
| PLXNA4 | 27.2(12.4-32.8) | 28.9(1.3-42.1) | 0.887010162395657 |
| CXADR | 1.3(0.1-21.4) | 1.5(-0.1-25) | 0.888750517951871 |
| EIF4G1 | 25.8(15.4-62.3) | 23.9(11.8-65.2) | 0.888996867243051 |
| PRKAB1 | 6.7(-1.3-21.5) | 3.4(-1.6-23.5) | 0.891151489630746 |
| PDGFB | 58.6(52.7-64.7) | 59.4(51.5-64.9) | 0.898038364744908 |

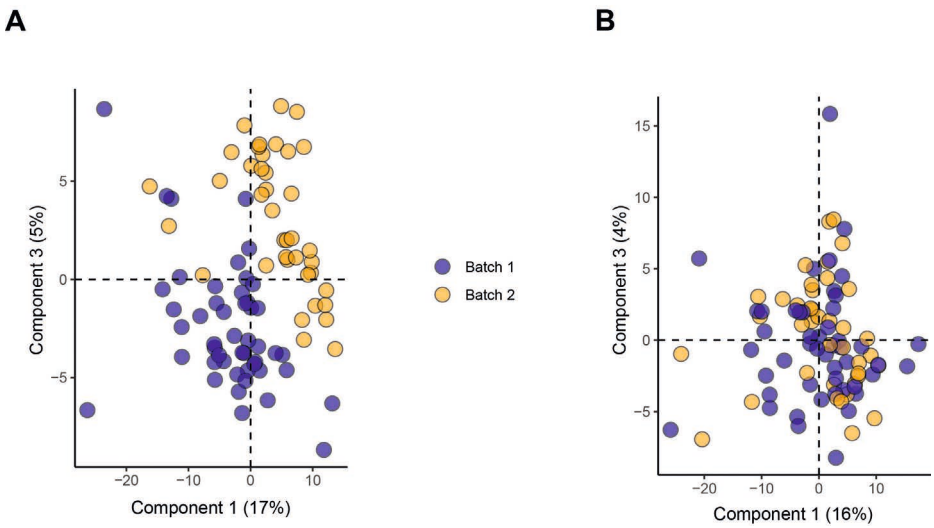
Supplementary table 3: Continued

| Protein | Responder | Non-responder | pvalue |
|---|---|---|---|
| TNFSF12 | 17(11.5-22.7) | 17.6(14.4-21.7) | 0.900236686199385 |
| TNFSF13 | 11.8(-3.5-18.5) | 12.5(-3.5-18.4) | 0.900630143889835 |
| FABP1 | 2.5(-24.3-21.3) | -1.2(-23.6-23.6) | 0.90077465429976 |
| ITM2A | 9.6(-1.1-20.8) | 9.6(-1.8-22.7) | 0.902843739097917 |
| MGMT | 23.9(14.9-52.8) | 21.6(4.9-60.8) | 0.903607162748807 |
| SHMT1 | 2.5(-2.3-16.5) | 2.9(-12.5-31.7) | 0.903732380014395 |
| CXCL12 | 15(3.5-21.9) | 13.8(-2.2-23.5) | 0.906424326843598 |
| SERPINB8 | 7.6(5.7-20.5) | 7.8(-2.6-22.4) | 0.91007469105426 |
| NRTN | -0.2(-9.9-0.6) | 0.3(-15.5-0.5) | 0.911365428302264 |
| SCRN1 | 1.3(-1.2-22.8) | 1.3(-0.7-28.6) | 0.912284616154511 |
| MLN | 2(-31.7-17) | 1.9(-15.7-15.1) | 0.914068219516405 |
| IL18R1 | 0.7(-0.2-14.8) | 0.6(0.2-15.3) | 0.91422461429078 |
| FCRL2 | 0.6(-0.5-19.1) | 0.9(0-14) | 0.91479351528653 |
| CCL3 | 13.1(-1.9-25.1) | 13.7(-0.9-24.8) | 0.921982345723019 |
| TPP1 | 18.8(14.9-26.9) | 18.5(11.6-27.9) | 0.924683959957881 |
| MAPK9 | 1.1(-0.1-15.1) | 0.9(0-22) | 0.925244347585569 |
| IL10 | -52.5(-60.3--37.2) | -51.8(-63.7--36.9) | 0.925963550364827 |
| CD48 | 1.4(0-14.3) | 1.6(-0.1-14.8) | 0.927437254349047 |
| CXCL3 | 46.9(25.9-63.9) | 42.3(34.8-73.3) | 0.930982733772942 |
| ARHGEF12 | 15.6(10.1-46.5) | 16(7.9-38.3) | 0.93250600157336 |
| TIMP3 | 65.7(48.2-98.4) | 67.5(49.3-84.3) | 0.933243954886508 |
| IL15RA | 1.1(-0.2-16.4) | 0.9(-0.6-16.4) | 0.934075812965402 |
| SPRY2 | 2.8(-0.2-17) | 2.8(-1-17.7) | 0.936705677883976 |
| PSIP1 | 11.3(5.2-29.9) | 13.9(-2.4-38.3) | 0.952157774480628 |
| TRIM21 | 10.9(-0.7-30.4) | 10.1(-0.7-42.8) | 0.954350890178984 |
| CD58 | 0.5(-0.5-1.1) | 0.5(0-1.2) | 0.957260527765761 |
| IL15 | 0.6(-0.2-11.2) | 0.6(-0.3-12.7) | 0.958932668946676 |
| HLA-DRA | 0.5(-0.3-22.7) | 1.1(-0.2-17.6) | 0.961145877314793 |
| CCL17 | 46.9(38.3-80.3) | 49.2(34.6-66.1) | 0.962833124434282 |
| METAP1D | 0.2(-0.7-26.4) | -0.1(-0.7-18.2) | 0.966544816956178 |
| IL7 | 33.6(25.2-49.7) | 33.7(23.7-41.6) | 0.972603762010356 |
| CHRDL1 | 0.2(-0.8-1.1) | 0.2(-9.4-10.9) | 0.972870864414606 |
| IFNG | 11.3(-21.9-29.9) | 0.5(-20.3-55.4) | 0.978318930102797 |
| FOXO1 | 8.6(5.5-36.3) | 9.2(-3.5-39) | 0.98281643093774 |
| ARTN | 0.1(-0.4-14.6) | 0.2(-0.4-14.5) | 0.983489826509922 |
| TNFAIP8 | 0.5(-0.3-11.3) | 0.4(-0.1-13.6) | 0.991770206140538 |
| DFFA | 21.4(6.3-33.3) | 20(6.9-44.7) | 0.994874587981631 |
| STX8 | 1(-0.7-30.8) | 0.7(-0.9-21.5) | 0.995578033001121 |
| ICA1 | 15.7(-2.1-29.2) | 12.1(-2.1-38.1) | 0.998423671250991 |
| IRAK4 | 23.3(7.2-47.7) | 19.5(6.8-61.5) | 0.99962369072898 |

**Supplementary table 4:** Expression data of the top ten proteins between responders (n=18) and non-responders (n=19) and between cluster 1 (n=17) and cluster 2 (n=20). Normalized Protein expression (median [min-max]).

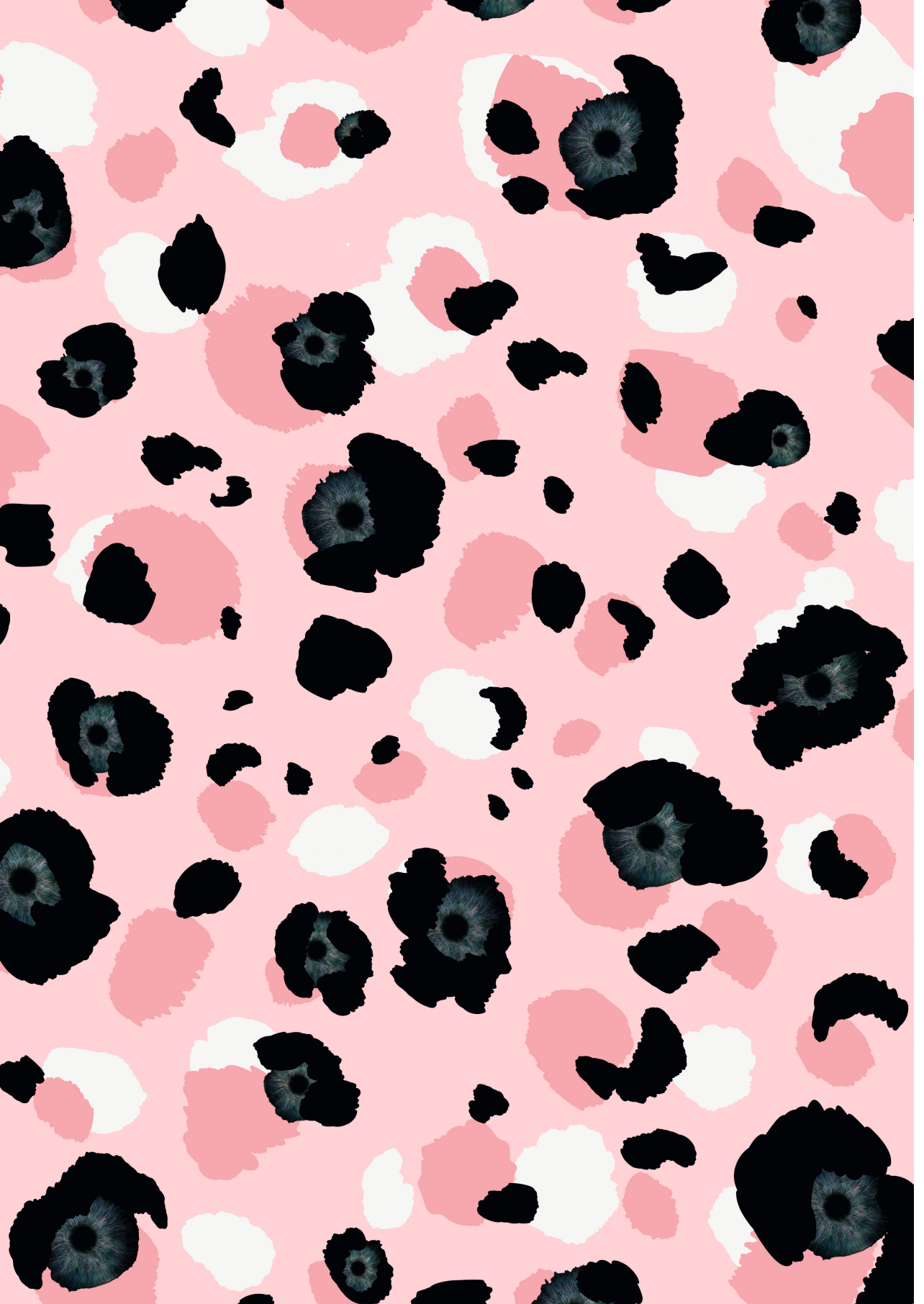
| Protein | Responder | Non-responder | Cluster 1 | Cluster 2 |
|---|---|---|---|---|
| ANGPT1 | 51.1(30.9-54.1) | 47.9(41.5-51.4) | 48(41.5-51.4) | 51(30.9-54.1) |
| BTN2A1 | 1.5(-0.4-13.6) | 0.5(-0.1-1.6) | 1.2(-0.1-1.6) | 1.3(-0.4-13.6) |
| CKAP4 | 12.2(0.4-26.4) | 15.4(10.3-20.4) | 15.6(10.3-26.4) | 11.7(0.4-16.8) |
| CLEC4C | 16(1.1-28.1) | 12.2(0.5-19.4) | 12.3(0.5-19.4) | 16(11.2-28.1) |
| CXCL17 | -5.1(-17-1) | -3.3(-13.1-18.3) | -3.4(-13.1-18.3) | -4.1(-17-1) |
| LRRN1 | 0.9(-0.3-14) | 11.6(0.2-16.2) | 12.1(0.7-16.2) | 0.8(-0.3-12.9) |
| MEGF10 | 12.3(-0.4-20.3) | 15.9(-0.2-22) | 16.7(14.2-22) | 11.7(-0.4-19.8) |
| OMD | 13.1(-0.8-21.4) | 17.3(2.3-20.2) | 17.8(14.8-20.2) | 12.6(-0.8-21.4) |
| OSM | 33.9(3.4-48.3) | 23.9(10.3-39.7) | 28.3(10.3-40.1) | 27.9(3.4-48.3) |
| SIRPB1 | 15.7(1.4-23.6) | 2(-0.1-19.3) | 2(-0.1-19.3) | 14.7(0.1-23.6) |

## Supplementary figures




**Supplementary figure 1**. Principal component analysis of overall serum proteomic signature (n=368 proteins) before and after batch correction.

**A)** Principal component analysis before batch correction **B)** Principal component analysis after batch correction using *ComBat* method.[36]



# CHAPTER 6

## Whole transcriptome analysis reveals heterogeneity in B cell memory populations in patients with juvenile idiopathic arthritis-associated uveitis

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## ABSTRACT

**Purpose:** Patients with juvenile idiopathic arthritis (JIA) are prone to developing chronic anterior uveitis (JIA-U+). Although several risk factors for JIA-U+ have been identified, the underlying etiology is poorly understood. Histopathological studies demonstrate B cell infiltrates in eye tissues of patients with JIA-U+.

**Methods:** We performed transcriptome profiling of peripheral blood CD19-positive B cells taken from 14 cases with JIA-U+, 13 JIA cases without uveitis (JIA-U-), and five healthy controls. Deconvolution-based estimation was used to determine the immune cell fractions for each sample.

**Results:** Deconvolution results revealed that naive B cells made up on average 71% of the CD19-positive cell fractions analyzed. Differential expression analysis identified 614 differentially expressed genes (DEGs) between the groups at nominal significance and six genes at a false discovery rate of 5% (FDR<0.05). Head-to-head comparison of all JIA-U- versus JIA-U+ revealed no DEGs in the CD19+ B cell pool (FDR<0.05). However, principal component analysis based on a panel of key genes for B cell subsets revealed that JIA-U+ cases bifurcate into distinct clusters, characterized by markedly disparate expression for genes associated with specific memory B cell populations. CIBERSORT analysis of the overall transcriptome of the new uveitis cluster identified an increased proportion of memory B cells.

**Conclusion:** These data show that JIA-U- and JIA-U+ have a globally similar transcriptome considering the global peripheral CD19-positive B cell pool. However, heterogeneity in B cell memory genes among cases with uveitis suggests a role for specific memory B cell subsets in the etiology of JIA-U+.

## INTRODUCTION

Chronic anterior uveitis (CAU) is a common feature of oligo- and poly-articular juvenile idiopathic arthritis (JIA) that leads to unilaterally blindness in 6-24% of the children and 33% of the eyes have become visually impaired in adulthood.[1-3] Although the incidence of uveitis in JIA varies between studies, approximately one third of JIA cases typically develop CAU within four years after JIA onset.[4,5] JIA-associated uveitis (JIA-U+) is often characterized by an insidious onset with a high risk for developing visually-threatening complications. Consequently, JIA-U+ warrants intensive monitoring and requires specialized ophthalmological care.

It remains highly challenging to predict the onset of uveitis in advance. However, advances in clinical and molecular profiling studies have helped in better understanding which children are particularly prone to developing uveitis.[6] A recent genome-wide association study highlighted distinct genetic susceptibility for uveitis in JIA.[6,7] JIA cases with uveitis more often are anti-nuclear antibody (ANA) positive and have increased levels of erythrocyte sedimentation rate (ESR) and S100A12 (calcium-binding protein).[6] Recently, flow cytometry studies have linked changes in blood T cells and monocytes to uveitis in JIA.[8,9] In contrast, immunohistochemical studies from iris biopsies and enucleated eyes revealed predominately infiltrating plasma cells and CD20-positive B cells.[10,11] The contribution of B cells in the pathophysiology of JIA-U+ is supported by the observation that anti-CD20 monoclonal antibody therapy (Rituximab) is effective in treating patients with (severe) JIA-associated uveitis.[12,13] The association of ANA with uveitis in JIA also supports that B cell hyperactivity contributes to the development of uveitis in cases with JIA.[6,14,15] Here, we report on the investigation of the transcriptome of peripheral blood B cells of cases with JIA and JIA-associated uveitis.

## METHODS

**Patients and patient material**

The study was approved by the Medical Ethical Research Committee in Utrecht in concordance with the Helsinki principles. Written informed consent was obtained from all 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.

We collected heparinized venous blood from a total of 32 children (≤16 year) with juvenile idiopathic arthritis with uveitis, (JIA-U+, n=14), and children with JIA without uveitis (JIA-U-, n=13) and healthy controls (HC, n=5) visiting the ophthalmologist or the pediatric rheumatologist at the University Medical Center Utrecht in the Netherlands. The JIA diagnosis was confirmed by a pediatric rheumatologist based on the criteria of the International League of Associations of Rheumatology.[16] All patients were screened by an ophthalmologist specialized in childhood uveitis according to the guidelines of the Academy of Pediatrics.[17] The patients with JIA-U- had an ophthalmologic follow-up of at least four years without signs of uveitis. JIA-associated uveitis was diagnosed according to the Standardization of Uveitis Nomenclature (SUN) criteria.[18] All patients had active uveitis at time of sampling. None of the patients received immunomodulatory treatment other than methotrexate (**Table 1**). The Wilcoxon rank sum and Kruskal-Wallis test were used to assess group differences for continuous variables and Fisher's Exact Test, Pearson's Chi-square test for categorical variables. *P*-values below 0.05 were considered nominal significant.

**B cell isolation**

For each case peripheral blood mononuclear cells (PBMCs) were isolated by standard ficoll gradient centrifugation from 9 mL heparinized blood immediately after blood withdrawal and subjected to sorting by The BD FACSAria™ III sorter after incubation with antibody-conjugated surface antibodies (**Supplementary table 1**) and FACS buffer (1% bovine serum albumin and 0.1% sodium azide in phosphate buffered saline) and blood lymphocytes purified ($CD14^-CD3^+CD19^+$) peripheral (PBL) were stored in liquid nitrogen for later analysis. PBL samples were thawed in batches of 4-8 samples, divided over five days, quickly thawed, washed with ice cold phosphate buffered saline and stained using the fluorescently-conjugated antibodies in **Supplementary table 2**. $CD45^+CD3^-CD19^+$ cells were sorted using The BD FACSAria™ III sorter. An example of the gating strategy is provided under **Supplementary Figure 1**.

**RNA sequencing**

FACS purified CD19$^+$ B cells were immediately taken up in lysis buffer (RLT plus, Qiagen, Venlo, Netherlands) containing 1% β-mercaptoethanol and subjected to RNA extraction using the AllPrep Universal Kit (Qiagen) on the QIAcube according to the manufacturer's instructions. In three samples the RNA was from insufficient quality and these samples were retained from further analysis. cDNA libraries were generated by GenomeScan (Leiden, the Netherlands) with the TruSeq RNAseq RNA Library Prep Kit (Illumina Inc., Ipswich, MA, USA), and were sequenced using Illumina HiSeq 4000 generating ~20 million 150bp paired ended reads for each sample.

**Differential gene expression**

FastQC tool was used for the quality check of the raw sequences. Reads were aligned to the human genome using STAR aligner[19] and Python package HTSeq[20] was used to count the number of reads overlapping each annotated gene. Count data were fed into DESeq2[21] to conduct differentially expression analysis. Subsequently, DESeq2 was used to model the biological variability and overdispersion in the gene expression data as a negative binomial distribution. We used Wald's test to identify DEGs in pairwise comparison and used likelihood ratio test (LRT) to identify DEGs considering multiple disease groups. We used variance stabilizing transformation (vsd) to plot normalized gene counts. We corrected *P* values using a false discovery rate (FDR) of 5% according to the Benjamini and Hochberg method.

**Principal component analysis**

We extracted expression data for 13 marker genes that distinguish peripheral blood B cell subsets as determined by single-cell sequencing analysis[22] and also include genes encoding IgA, IgM, and IgG, (17 genes in total, see **Supplementary Table 3**), because previous studies have linked immunoglobulin expression to juvenile idiopathic arthritis-associated uveitis.[23] Differences in the expression of these genes was considered to indicate changes in (memory) B cell subsets in blood, which would be evident by using this set of genes to conduct principal component analysis with the *factoextra* package in R. Using the first two principal components, we reclassified patients into new groups. We next used deconvolution-based estimation of memory B cell fractions (see below) and differential expression analysis by DESeq2[21] to support if the PCA-identified clusters of patients were genuinely characterized by changes in gene expression. Expression data (median TPM values) for relevant B cell genes in

purified blood naive B cells, non-switched B cells, and switched B cells as determined by RNA sequencing was obtained from Monaco et al.[24]

**Deconvolution-based estimation of cell fractions**

For deconvolution of immune cell composition per patient sample, the gene expression data were analyzed by CIBERSORT using the standard reference signature expression matrix for leukocytes (LM22)[25]. Kruskal-Wallis test was used to assess group differences for estimated B cell populations in cell fractions.

## RESULTS

Patient characteristics are shown in **Table 1**. From a total of 27 patients with JIA, 20 patients (74.1%) were diagnosed with oligoarticular subtype, 6 patients with rheumatoid factor – negative polyarthritis (22.2%). Among the JIA patients with uveitis, 10 (71%) were classified with the oligoarticular subtype and 3 (21%) patients with rheumatoid factor-negative polyarthritis. As expected, patients with uveitis were more often ANA-positive and had a lower age of onset of JIA compared to patients without uveitis (**Table 1**).

We purified the blood ($CD14^-CD3^-$) $CD19^+$ B cell population by flow cytometry from JIA cases with and without uveitis and controls, and performed whole-transcriptome RNA sequencing. After quality control, a total of 29 samples were used to investigate the transcriptomic signature of blood B cells. The $CD19^+$ lymphocyte fraction comprises several B cell populations, including naive and memory B cells. Therefore, we first estimated the immune-cell composition from the bulk transcriptomic data using deconvolution-based estimation by CIBERSORT.[25] As expected, the relative fraction of B cells was high (>80%) across all samples (**Figure 1A**). Naive B cells made up the majority of the fraction (mean across all samples 71%), followed by memory B cells (mean, 13%), and several other lymphocytes populations (**Figure 1A**). The estimated non-B cell populations most likely represents spill over caused by a subset of marker genes from the signature matrices also expressed in other lymphocytes and therefore display lower cell-specificity.[26] Regardless, we observed no evidence for changes in the memory or naive B cell subsets between the disease groups (Kruskal-Wallis test, $P = 0.34$ and $P = 0.69$, **Figure 1B**).

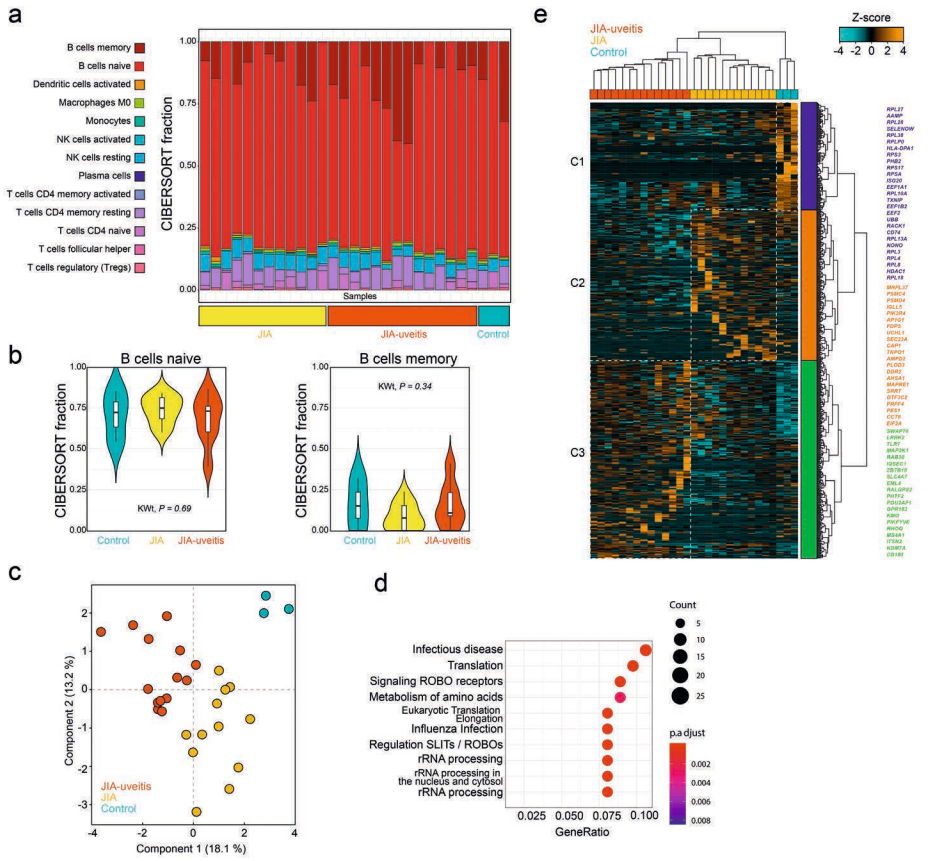


**Figure 1.** Blood CD19⁺ B cell transcriptomics in juvenile idiopathic arthritis-associated uveitis.

**A)** Deconvolution-based estimation of immune cell fractions using the RNA-sequencing data and CIBERSORT.[25] The estimated cell fraction for each immune cell type in each sample is depicted in a stacked barplot (using the LM22 as signature sets to estimate the cell fractions) in the disease groups and controls. **B)** The estimated cell fraction for naive and memory B cells for each of the disease groups and Kruskal-Wallis test statistic (KWt). **C)** Principal component analysis of 614 DEGs at nominal significance (likelihood ratio test $P$ <0.05). **D**) Pathway enrichment analysis of the 614 DEGs using the R package *clusterProfiler* with the *Reactome* database. **E)** Hierarchical clustering of the 614 DEGs from the **Figure 1C** using Euclidean distance and Ward's method. Cluster 1-3 are indicated by different colors. A selection of genes associated to each cluster is indicated by the corresponding color (full list of genes and clusters are depicted in **Supplementary table 4 and 6**).

**Table 1.** Characteristics of the cohort investigated in this study.

| | JIA | HC | JIA-U- | JIA-U+ | *P-value* |
|---|---|---|---|---|---|
| N | 27 | 5 | 13 | 14 | NA |
| Male, n (%) | 8 | 1 (20) | 4 (31) | 4 (29) | 1.000 |
| Age in years, median (IQR) | 12 (9-19) | 26 (26-27) | 14 (10-19.5) | 11 (8-19) | 0.002 |
| Methotrexate therapy, n (%) | 16 (59%) | NA | 6 (46) | 10 (71) | 0.182 |
| ANA positivity, n (%) | 20 (74%) | NA | 7 (54) | 13 (93) | 0.033 |
| Age at uveitis diagnosis, median (IQR) | 6 (4-8) | NA | NA | 6 (4-8) | NA |
| Age at arthritis diagnosis, median (IQR) | 6 (2-9) | NA | 8 (4.5-10) | 2 (1-6.5) | 0.005 |
| Duration of uveitis in years, median (IQR) | 5 (3-10) | NA | NA | 5 (3-10) | NA |

HC, healthy controls; JIA-U-, juvenile idiopathic arthritis without uveitis; JIA-U+, juvenile idiopathic arthritis with uveitis; ANA, anti-nuclear antibody; N.A., not applicable; IQR, interquartile range.

Next, the transcriptomic data was subjected to differential expression analysis, which revealed 614 differentially expressed genes (DEGs) at nominal significance between the groups (likelihood-ratio test, $P$ <0.05) and six DEGs at FDR of 5% (**Figure 1C,** and **Supplementary table 4 and 5)**. Results of the pathway enrichment analysis for the 614 DEGs is shown in **Figure 1D**. Hierarchical cluster analysis of the 614 DEGs discerned three overarching clusters labelled C1 to C3 (**Figure 1E**). The gene signatures of each of the three clusters typically corresponded with one of the investigated disease groups. The first cluster (C1) contained mostly genes of the ribosomal machinery (i.e., *RPL* and *RPS* genes, **Figure 1E**) and was relatively high expressed in controls. This cluster also contained uveitis risk gene *HLA-DPB1,* which was decreased in JIA-U+ cases ($Log_2FC$= -0.33, $P$ = 0.016) and JIA cases (JIA-U-) compared to controls ($Log_2FC$=-0.34, $P$ = 0.013). The second cluster (C2) of genes was relatively higher expressed in JIA cases without uveitis and contained genes involved in cholesterol biosynthesis and mevalonate pathway (e.g. *MVD, FDPS, SEC23A*). Cluster 3 (C3) represented a gene signature associated with uveitis that includes core B cell genes, including the *MS4A1* gene (encoding CD20), Toll-Like Receptors (TLR) *TLR7* and *CD180* that regulates B cells responses, and *ITSN2* a key gene required for antibody formation in B cells (**Supplementary table 6**).

**Bulk B cell transcriptome reveals heterogeneity in JIA cases with uveitis**

Head-to-head comparison of JIA cases with and without uveitis revealed 387 DEGs at nominal significance (**Supplementary table 7**) and no genes at a FDR of 5%. However, recent single cell analyses has revealed that the peripheral blood B cell compartment contains ~10 functionally distinct B cell subsets.[22,27] Because the peripheral B cell fraction is dominated by naive B cells (**Figure 1A**), we hypothesized that B cell subset-

specific gene expression from relatively rarer cell types in blood, may be drowned out in bulk transcriptome analysis. To this end, we assessed the relative expression of 17 marker genes previously associated with individual B cell subsets in peripheral blood mononuclear cells (**Supplementary table 3**). Principal component analysis based on the panel of B cell subset genes revealed that three cases displayed relatively higher levels for Immunoglobulin G and A genes (**Figure 2A**). In addition, PCA analysis revealed heterogeneity among cases with uveitis. Overall, distinct clusters of uveitis cases (termed "JIA uveitis 2") became apparent, characterized by distinct expression of *IGHD, CCR7, IGHM and CD27* (**Figure 2B**). Indeed, differential expression analysis based on the reclassification into three groups (JIA, JIA-uveitis 1, and JIA-uveitis 2, see **Figure 2A**) identified 41 genes at a FDR of 5%, including *IGHD* (LRT, *Padj* = $2.1 \times 10^{-3}$) and *CCR7* (*Padj* = $3.7 \times 10^{-2}$) and *KCNN4* (LRT, *Padj* = $4.2 \times 10^{-6}$), and *BAIAP3* (LRT, *Padj* = $1.3 \times 10^{-4}$) genes (**Supplementary table 8**). Gene expression profiles for these genes associated with the distinct cluster of JIA-uveitis cases are reminiscent of the (IgD-, IgM-) switched memory B cells (**Figure 2C**).[24,28] Gene set enrichment analysis revealed that genes associated with the reclassification of uveitis cases are associated with GO terms; *lymphocyte activation* (GO:0046649, *Padj* = $3.3 \times 10^{-5}$), *B cell activation* (GO:0042113, *Padj* = $9.2 \times 10^{-4}$), and *immune response-activating cell surface receptor signaling pathway* (GO:0002429, *Padj* = $9.1 \times 10^{-4}$). To assess if the overall transcriptome of the newly identified uveitis cluster was indeed associated with memory B cells, we reanalyzed the B cell fractions using the signature genes of CIBERSORT (n=547 genes). This analysis revealed that the identified subgroup of uveitis cases (JIA-uveitis 2 in **Figure 2A**) is characterized by a significantly increased proportion of memory B cells, coupled with a decrease in naive B cells compared to the other JIA groups (KW-test; *P* = 0.0019, *P* = 0.0027, respectively, **Figure 2D**). Baseline characteristics of the distinct uveitis clusters are presented in **Table 2**.

Although age-related and disease course associated changes in memory B cells have been reported[29,30] we did not observe a correlation between memory or naive B cell population and age or disease duration (**Supplementary figure 2**). We observed a relatively higher estimated memory B cell fraction in JIA cases with late-onset uveitis, but this difference was not statistically significant (*P* = 0.08) (**Supplementary figure 3**).

Finally, head-to-head-comparison of the two newly identified JIA-uveitis clusters revealed 485 genes at a FDR of 5% (**Supplementary table 9**). These included, as expected, *IGHD* (*Padj* = $9.4 \times 10^{-5}$), *KCNN4* (*Padj* = $9.5 \times 10^{-8}$), and *BAIAP3* (*Padj* = 2.2

× $10^{-6}$) (**Figure 2E**), but also revealed differences in the expression of key receptors implicated in B cell immunity such as cytokine receptors *IL4R* (*Padj* = 1.2 × $10^{-4}$), *IL21R* (*Padj* = 5.8 × $10^{-3}$), *TNFRSF13B* (*Padj* = 0.03), the receptor for B cell cytokines APRIL and BAFF, and *SCIMP* (*Padj* = 5.8 × $10^{-3}$), a transmembrane protein involved in major histocompatibility complex class II (i.e., HLA-DR) signaling (**Figure 2F**).

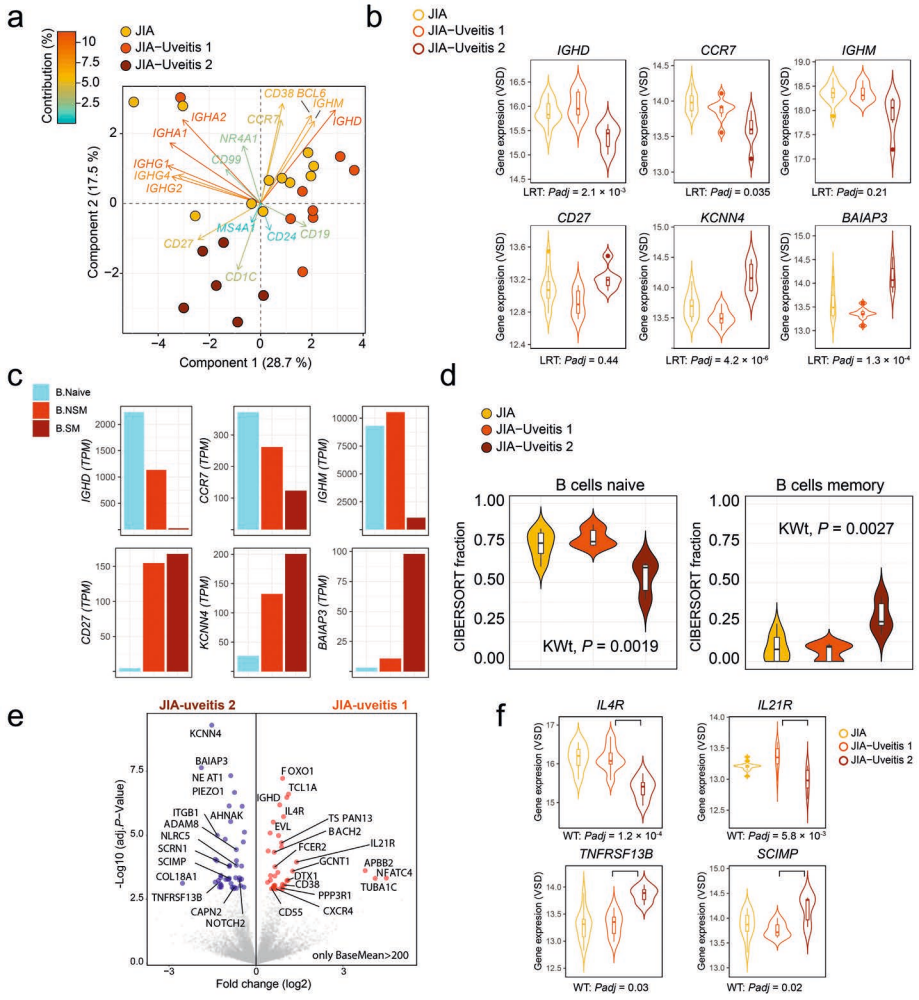


**Figure 2.** Heterogeneity in key B cell memory genes in JIA cases with uveitis.

**A)** Principal component analysis biplot of JIA cases with and without uveitis based on the expression data of 17 genes associated with B cell subsets. The contribution (in %) of each gene to the components is indicated. Two subgroup of uveitis cases are indicated in dark red and orange. **B)** Gene expression for *CCR7, CD27, KCNN4, BAIAP3, IGHM,* and *IGHD* genes in JIA and the two JIA-uveitis clusters. LRT; likelihood ratio test from DESeq2 considering the JIA and the two JIA-uveitis clusters, Padj; FDR corrected *P* values. **C)** The median gene expression (TPM values) for genes of **Figure 2B** in Naive B cells (B Naive), non-switched memory B cells (NS Mem), and switched memory B cells (S mem) from Monaco et al.[24] **D)** Deconvolution-based estimation by CIBERSORT[25] of naive and memory B cells fraction for JIA and the two JIA-uveitis groups identified by the PCA analysis under **Figure 2A**. KWt; Kruskal-Wallis test. **E)** Volcano plot of differentially expressed genes (DEG) from the head-to-head comparison of the JIA-uveitis cluster 1 versus JIA-uveitis cluster 2 (see also **Figure 2A**). The 10,859 high expressed genes (BaseMean>200 from DESeq2) are indicated in grey. DEGs are indicated in red (increased expression) and blue (decreased in expression). **F)** Gene expression for *IL4R, IL21R, SCIMP,* and *TNFRSF13B* in JIA and the two JIA-uveitis clusters. WT: Wald's test from DESeq2 considering head-to-head comparison of the newly identified two JIA-uveitis clusters, Padj; FDR corrected *P* values.

**Table 2.** Characteristics of the two distinct uveitis clusters

| | JIA | HC | JIA-U- | JIA-U+ | *P-value* |
|---|---|---|---|---|---|
| N | 27 | 5 | 13 | 14 | NA |
| Male, n (%) | 8 | 1 (20) | 4 (31) | 4 (29) | 1.000 |
| Age in years, median (IQR) | 12 (9-19) | 26 (26-27) | 14 (10-19.5) | 11 (8-19) | 0.002 |
| Methotrexate therapy, n (%) | 16 (59%) | NA | 6 (46) | 10 (71) | 0.182 |
| ANA positivity, n (%) | 20 (74%) | NA | 7 (54) | 13 (93) | 0.033 |
| Age at uveitis diagnosis, median (IQR) | 6 (4-8) | NA | NA | 6 (4-8) | NA |
| Age at arthritis diagnosis, median (IQR) | 6 (2-9) | NA | 8 (4.5-10) | 2 (1-6.5) | 0.005 |
| Duration of uveitis in years, median (IQR) | 5 (3-10) | NA | NA | 5 (3-10) | NA |

HC, healthy controls; JIA-U-, juvenile idiopathic arthritis without uveitis; JIA-U+, juvenile idiopathic arthritis with uveitis; ANA, anti-nuclear antibody; N.A., not applicable; IQR, interquartile range.

## DISCUSSION

We performed transcriptome analysis of peripheral blood CD19$^+$ B cells in JIA-U- and JIA-U+ patients. Our analysis suggests that the peripheral blood CD19$^+$ B cells pool in JIA-U- and JIA-U+ patients display an overall remarkably similar transcriptome, also compared to controls. The differential expression analysis revealed only six differently expressed genes (DEGs) after FDR correction, which suggests that these differences are unlikely robust, but included genes such as *TXNIP* - related to of B cell associated germinal centers in peripheral lymphoid organs[31] and *MN1*, linked to colony forming activity of B cells.[32] Polymorphisms (SNPs) in the *HLA-DPB1* gene are associated with the susceptibility to uveitis in JIA[7], but the gene expression of *HLA-DPB1* was (slightly) decreased in JIA cases either with or without uveitis compared to controls (JIA-U+, Log2[FC]= -0.33, $P$ = 0.016 ; JIA-U-, Log2[FC]= -0.34, $P$ = 0.013). This can be explained by the fact that the variant associated with uveitis (rs6457109 in *HLA-DPB1*) is not an expression quantitative trait loci[33] for this gene, and thus, not associated with its expression, but predominantly with its peptide binding capacity.

We further show that, as expected, transcriptomic deconvolution revealed a large fraction of naive B cells in the total peripheral pool of CD19+ cells. This suggests that future analysis should focus on subsets beyond naive cells in JIA, for example using single cell RNA sequencing or high dimensional cytometry. Despite this limitation, this study may guide future studies into relevant B cell subsets. We exploited marker genes used to mark B cell subsets in peripheral blood mononuclear cells in recently reported single-cell studies.[22,27] We would like to emphasize that this set

of genes is by no means exhaustive, but showed to be instrumental in our study to reclassify cases, because we showed that the newly identified subgroup of JIA-uveitis cases displayed genuine enrichment for memory B cell gene circuits as supported CIBERSORT analysis and distinct gene profiles as shown by differential expression analysis. Using this strategy, we identified a previously underappreciated heterogeneity among cases with uveitis that is characterized by increased proportion of memory B cells, most likely switched memory B cell populations. This is of interest, because in young patients with early-onset oligoarticular JIA (a risk factor for uveitis) switched memory (CD27+IgM-IgD-) B cells are expanded in blood and associated with the production of anti-nuclear antibodies (ANAs).[29] B-cell memory subsets that express relatively lower CCR7 are also increased in synovial fluid from oligoarticular JIA patients, suggesting that these cells also play a role in arthritis.[34] However, a recent flow cytometry study revealed a decrease in naive B cells, coupled with an increase in switched B cells, and memory B cells in a subset of JIA patients.[35] In light of the current study, these observations make it tempting to speculate that the changes in B cell composition in blood of JIA may in part be related to the presence or susceptibility to the development of uveitis. Considering uveitis as a multifactorial disease it is possibly mediated by redundant disease mechanisms. For example, increased circulating B cell memory may contribute significantly in a part of cases, while other factors may dominate in other cases. This is also reflected in clinical observations such as anti-nuclear antibodies, a risk factor for uveitis, that are more common – but not detected in all- uveitis cases. Although our study lacked sufficient power to investigate the association of the B cell signatures with clinical uveitis subgroups, genetic studies demonstrated that uveitis in JIA is genetically heterogeneous and may require reclassification into mechanistic disease endotypes.[7]



Notably, however, post-switch $IgD^{-}CD27^{+}$ B cells increase with longer disease duration in a study with rheumatic arthritis patients.[36] This of interest, because clinical data of patients in this study suggest comparable trends, with cases in JIA-uveitis 2 showing longer disease duration compared to the other groups. In contrast, in B cell-mediated inflammatory conditions such as Sjogren's disease longer disease duration is associated with a decrease in memory B cell subsets and a more active disease profile.[37] Furthermore, although age-related changes in memory B cell subsets have been reported, the absolute number of peripheral blood naive and memory B cell subsets both decline from ±2-4 years old until adulthood.[30] This suggest that although the JIA-uveitis 2 group was on average older, the increase in memory B cell

subsets in these cases is unlikely merely attributable to differences in age. Indeed, we also did not observe a correlation of memory B cell count and age in our cohort (**Supplementary figure 2**).

In contrast, expansion of switched memory B cells has been linked to early-onset of JIA presenting before age 6 years, which persisted throughout the disease course in these cases.[29] The early-onset of JIA is also a risk factor for uveitis, which would make it tempting to speculate that the early-onset associated expansion of memory B cells is linked to uveitis development. In this small cohort, we did, however, not see a larger memory B cell gene fraction in early-onset JIA (**Supplementary figure 3**). This follows the work from Marasco and associates[29] who also did not find an association between JIA early-onset related memory B cell expansion and uveitis. Our data suggest that this is the result of heterogeneity in the B cell compartment of JIA cases with uveitis. Although underpowered, we found suggestive evidence that cases with increased memory B cell signatures can be characterized by late-onset of uveitis (**Supplementary figure 3**). We recommend further investigation into memory B cells in late-onset and early-onset uveitis in cases with JIA to dissect the contribution of these immune cells in the pathophysiology of uveitis in JIA.

Some cases in this study used methotrexate. Although previous studies have shown that IgG-positive memory B cell frequency is increased in JIA patients treated with methotrexate, the absolute numbers of memory B cells are not affected[38], nor did methotrexate halt the progressive increase of memory B cells in early-on set JIA.[29] Also here, we would like to emphasize that conclusive data on changes in the composition of B cell subsets warrants further in-depth cytometry analysis ideally at different time points in cohorts of JIA cases with significant ophthalmological follow-up. We propose that such future analyses are accompanied by single-cell sequencing approaches of peripheral blood immune cells or iris biopsies of JIA cases with uveitis.

To date, several studies support a role for B cells in the pathogenesis of JIA-U+. Small studies have revealed increased abundance of CD20+ B cells in eye tissues of cases with JIA-uveitis. In fact, plasma cells and $CD20^+$ B cells outnumbered other immune cells in enucleated eyes and iridectomy specimens.[10,11] A more recent study performed transcriptomic and proteomic analysis of iris tissue in three JIA-U+ and three primary open-angle glaucoma patients and detected increased expression for immunoglobulin genes and B cell-associated proteins in JIA-U+.[39] None of the

upregulated B-cell associated genes identified in this study were found to be different between the disease groups in our study. This could suggests discrepancies between peripheral blood and primary site of inflammation, however, both studies support a role for memory B cells in JIA-associated uveitis. Although the mechanisms of B cell mediated immune responses in JIA-U remain unknown, intraocular antibodies against Parvovirus B19 were significantly higher in JIA-U+ patients compared to children with other types of anterior uveitis, which may have triggered an immune response.[40]

In summary, we found no differences in transcriptome of the peripheral blood $CD19^{+}$ B cells pool in JIA-U- and JIA-U+ patients. However, dimension reduction-based analysis of B cell subset genes identified a distinct subset of JIA cases with uveitis characterized by the expression of memory B cell genes.

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# SUPPLEMENTARY DATA

## Supplementary tables

**Supplementary table 1**: Antibody panel used for sorting peripheral blood lymphocytes (CD14-CD3+CD19+)

| Marker | Fluorochrome | Dilution | Company |
|---|---|---|---|
| CD3 | AF700 | 1:25 | BioLegend |
| CD19 | AF700 | 1:50 | BD |
| CD4 | BV711 | 1:50 | Sony Biotechnology |
| CD8 | V500 | 1:50 | BD |
| CD14 | PerCP-Cy5.5 | 1:100 | eBioscience |

**Supplementary table 2:** Antibody panel used for sorting CD45+CD3-CD19+.

| Marker | Fluorochrome | Dilution | Company |
|---|---|---|---|
| CD3 | APC | 1:100 | BioLegend |
| CD19 | BV605 | 1:50 | BD |
| CD4 | BV711 | 1:00 | Sony Biotechnology |
| CD8 | V500 | 1:25 | BD |
| CD45 | PerCP | 1:100 | Sony Biotechnology |

**Supplementary table 3:** List of 17 marker genes previously associated with individual B cell subsets in peripheral blood mononuclear cells.

| Genes |
|---|
| *IGHA1* |
| *IGHA2* |
| *IGHG1* |
| *IGHG2* |
| *IGHG4* |
| *IGHM* |
| *IGHD* |
| *CD99* |
| *NR4A1* |
| *CCR7* |
| *CD38* |
| *BCL6* |
| *CD19* |
| *CD24* |
| *MS4A1* |
| *CD1C* |
| *CD27* |

**Supplementary table 4:** Differentially expressed genes (614) between healthy controls, JIA patients without uveitis and JIA patients with uveitis.

WEBLINK: https://www.frontiersin.org/articles/10.3389/fimmu.2020.02170/full#supplementary-material

**Supplementary table 5**: Differentially expressed genes (FDR 5%) between healthy controls, JIA patients without uveitis and JIA patients with uveitis.

| Gene (hgnc) | Ensemble gene ID | Log2Fold change | p-adj |
|---|---|---|---|
| TXNIP | ENST00000582401 | -1,16 | 9,50E+09 |
| CCND3 | ENST00000372991 | -1,42 | 0,00105488 |
| RPL24 | ENST00000464595 | -1,06 | 0,023940188 |
| TP53INP1 | ENST00000448464 | -0,51 | 0,023940188 |
| RHOQ | ENST00000465198 | 1,03 | 0,023940188 |
| RPLP0 | ENST00000392514 | -0,52 | 0,027280684 |

**Supplementary table 6:** List of 614 differentially expressed genes (LRT P<0.05) and the three overarching clusters from Figure 1E.

| Ensemble ID | Gene Name | Cluster |
|---|---|---|
| ENSG00000008517 | IL32 | 1 |
| ENSG00000019582 | CD74 | 1 |
| ENSG00000051523 | CYBA | 1 |
| ENSG00000061918 | GUCY1B3 | 1 |
| ENSG00000063177 | RPL18 | 1 |
| ENSG00000066923 | STAG3 | 1 |
| ENSG00000077150 | NFKB2 | 1 |
| ENSG00000080546 | SESN1 | 1 |
| ENSG00000084070 | SMAP2 | 1 |
| ENSG00000089157 | RPLP0 | 1 |
| ENSG00000100100 | PIK3IP1 | 1 |
| ENSG00000100227 | POLDIP3 | 1 |
| ENSG00000100316 | RPL3 | 1 |
| ENSG00000100353 | EIF3D | 1 |
| ENSG00000105401 | CDC37 | 1 |
| ENSG00000107521 | HPS1 | 1 |
| ENSG00000108107 | RPL28 | 1 |
| ENSG00000109906 | ZBTB16 | 1 |
| ENSG00000112576 | CCND3 | 1 |
| ENSG00000114391 | RPL24 | 1 |
| ENSG00000114942 | EEF1B2 | 1 |
| ENSG00000116478 | HDAC1 | 1 |
| ENSG00000122406 | RPL5 | 1 |
| ENSG00000124767 | GLO1 | 1 |

Supplementary table 6. (continued)

| Ensemble ID | Gene Name | Cluster |
|---|---|---|
| ENSG00000126353 | CCR7 | 1 |
| ENSG00000126822 | PLEKHG3 | 1 |
| ENSG00000127837 | AAMP | 1 |
| ENSG00000131469 | RPL27 | 1 |
| ENSG00000131652 | THOC6 | 1 |
| ENSG00000131724 | IL13RA1 | 1 |
| ENSG00000133835 | HSD17B4 | 1 |
| ENSG00000134962 | KLB | 1 |
| ENSG00000142534 | RPS11 | 1 |
| ENSG00000142541 | RPL13A | 1 |
| ENSG00000147140 | NONO | 1 |
| ENSG00000147403 | RPL10 | 1 |
| ENSG00000148795 | CYP17A1 | 1 |
| ENSG00000149091 | DGKZ | 1 |
| ENSG00000149273 | RPS3 | 1 |
| ENSG00000149557 | FEZ1 | 1 |
| ENSG00000150687 | PRSS23 | 1 |
| ENSG00000155962 | CLIC2 | 1 |
| ENSG00000156508 | EEF1A1 | 1 |
| ENSG00000156711 | MAPK13 | 1 |
| ENSG00000158856 | DMTN | 1 |
| ENSG00000161016 | RPL8 | 1 |
| ENSG00000162511 | LAPTM5 | 1 |
| ENSG00000162747 | FCGR3B | 1 |
| ENSG00000164587 | RPS14 | 1 |
| ENSG00000164938 | TP53INP1 | 1 |
| ENSG00000167601 | AXL | 1 |
| ENSG00000167658 | EEF2 | 1 |
| ENSG00000168028 | RPSA | 1 |
| ENSG00000168209 | DDIT4 | 1 |
| ENSG00000169184 | MN1 | 1 |
| ENSG00000170315 | UBB | 1 |
| ENSG00000170889 | RPS9 | 1 |
| ENSG00000171681 | ATF7IP | 1 |
| ENSG00000172116 | CD8B | 1 |
| ENSG00000172183 | ISG20 | 1 |
| ENSG00000172809 | RPL38 | 1 |
| ENSG00000173156 | RHOD | 1 |
| ENSG00000174444 | RPL4 | 1 |
| ENSG00000175061 | LRRC75A-AS1 | 1 |
| ENSG00000175130 | MARCKSL1 | 1 |
| ENSG00000178952 | TUFM | 1 |
| ENSG00000178980 | SEPW1 | 1 |
| ENSG00000182057 | OGFRP1 | 1 |
| ENSG00000182541 | LIMK2 | 1 |

Supplementary table 6. (continued)

| Ensemble ID | Gene Name | Cluster |
|---|---|---|
| ENSG00000182774 | RPS17 | 1 |
| ENSG00000185986 | SDHAP3 | 1 |
| ENSG00000186280 | KDM4D | 1 |
| ENSG00000186635 | ARAP1 | 1 |
| ENSG00000188693 | CYP51A1-AS1 | 1 |
| ENSG00000196159 | FAT4 | 1 |
| ENSG00000198435 | NRARP | 1 |
| ENSG00000198712 | MT-CO2 | 1 |
| ENSG00000198755 | RPL10A | 1 |
| ENSG00000198911 | SREBF2 | 1 |
| ENSG00000199568 | RNU5A-1 | 1 |
| ENSG00000199875 | | 1 |
| ENSG00000201863 | | 1 |
| ENSG00000204538 | PSORS1C2 | 1 |
| ENSG00000204628 | GNB2L1 | 1 |
| ENSG00000207440 | RNU6-541P | 1 |
| ENSG00000212297 | RNU6-821P | 1 |
| ENSG00000213131 | YWHAZP4 | 1 |
| ENSG00000213362 | FTH1P12 | 1 |
| ENSG00000213442 | RPL18AP3 | 1 |
| ENSG00000213553 | RPLP0P6 | 1 |
| ENSG00000213860 | RPL21P75 | 1 |
| ENSG00000214485 | RPL7P1 | 1 |
| ENSG00000215021 | PHB2 | 1 |
| ENSG00000215381 | | 1 |
| ENSG00000215861 | | 1 |
| ENSG00000220583 | RPL35P2 | 1 |
| ENSG00000220842 | | 1 |
| ENSG00000221949 | LINC01465 | 1 |
| ENSG00000223865 | HLA-DPB1 | 1 |
| ENSG00000224114 | | 1 |
| ENSG00000224668 | IPO8P1 | 1 |
| ENSG00000225178 | RPSAP56 | 1 |
| ENSG00000226828 | | 1 |
| ENSG00000228019 | | 1 |
| ENSG00000228205 | | 1 |
| ENSG00000228253 | MT-ATP8 | 1 |
| ENSG00000228292 | | 1 |
| ENSG00000228665 | | 1 |
| ENSG00000229251 | HNRNPA1P8 | 1 |
| ENSG00000229611 | | 1 |
| ENSG00000230667 | SETSIP | 1 |
| ENSG00000231389 | HLA-DPA1 | 1 |
| ENSG00000236552 | RPL13AP5 | 1 |
| ENSG00000240669 | | 1 |

Supplementary table 6. (continued)

| Ensemble ID | Gene Name | Cluster |
|---|---|---|
| ENSG00000241282 | RPL34P33 | 1 |
| ENSG00000241907 | RPS20P4 | 1 |
| ENSG00000243829 | | 1 |
| ENSG00000243896 | OR2A7 | 1 |
| ENSG00000244313 | | 1 |
| ENSG00000246526 | | 1 |
| ENSG00000248202 | | 1 |
| ENSG00000248774 | | 1 |
| ENSG00000249753 | | 1 |
| ENSG00000250222 | | 1 |
| ENSG00000253132 | IGHV3-62 | 1 |
| ENSG00000255725 | TDGP1 | 1 |
| ENSG00000255753 | | 1 |
| ENSG00000257616 | | 1 |
| ENSG00000259704 | | 1 |
| ENSG00000260572 | | 1 |
| ENSG00000260735 | | 1 |
| ENSG00000261612 | SUB1P3 | 1 |
| ENSG00000261744 | | 1 |
| ENSG00000262370 | | 1 |
| ENSG00000263846 | CIAPIN1P | 1 |
| ENSG00000265123 | RN7SL200P | 1 |
| ENSG00000265972 | TXNIP | 1 |
| ENSG00000266849 | | 1 |
| ENSG00000267534 | S1PR2 | 1 |
| ENSG00000268262 | | 1 |
| ENSG00000270503 | | 1 |
| ENSG00000271788 | | 1 |
| ENSG00000272386 | | 1 |
| ENSG00000272832 | | 1 |
| ENSG00000273406 | | 1 |
| ENSG00000275294 | | 1 |
| ENSG00000279117 | | 1 |
| ENSG00000279570 | | 1 |
| ENSG00000280985 | | 1 |
| ENSG00000281849 | | 1 |
| ENSG00000011009 | LYPLA2 | 2 |
| ENSG00000013275 | PSMC4 | 2 |
| ENSG00000013573 | DDX11 | 2 |
| ENSG00000038274 | MAT2B | 2 |
| ENSG00000040531 | CTNS | 2 |
| ENSG00000057252 | SOAT1 | 2 |
| ENSG00000065802 | ASB1 | 2 |
| ENSG00000067560 | RHOA | 2 |
| ENSG00000072682 | P4HA2 | 2 |

Supplementary table 6. (continued)

| Ensemble ID | Gene Name | Cluster |
|---|---|---|
| ENSG00000074706 | IPCEF1 | 2 |
| ENSG00000083312 | TNPO1 | 2 |
| ENSG00000087087 | SRRT | 2 |
| ENSG00000087266 | SH3BP2 | 2 |
| ENSG00000087903 | RFX2 | 2 |
| ENSG00000088298 | EDEM2 | 2 |
| ENSG00000090861 | AARS | 2 |
| ENSG00000091262 | ABCC6 | 2 |
| ENSG00000099204 | ABLIM1 | 2 |
| ENSG00000100029 | PES1 | 2 |
| ENSG00000100591 | AHSA1 | 2 |
| ENSG00000100934 | SEC23A | 2 |
| ENSG00000101367 | MAPRE1 | 2 |
| ENSG00000101997 | CCDC22 | 2 |
| ENSG00000103275 | UBE2I | 2 |
| ENSG00000104901 | DKKL1 | 2 |
| ENSG00000104921 | FCER2 | 2 |
| ENSG00000105219 | CNTD2 | 2 |
| ENSG00000105711 | SCN1B | 2 |
| ENSG00000106397 | PLOD3 | 2 |
| ENSG00000106686 | SPATA6L | 2 |
| ENSG00000108389 | MTMR4 | 2 |
| ENSG00000112280 | COL9A1 | 2 |
| ENSG00000115207 | GTF3C2 | 2 |
| ENSG00000116221 | MRPL37 | 2 |
| ENSG00000116337 | AMPD2 | 2 |
| ENSG00000117601 | SERPINC1 | 2 |
| ENSG00000118513 | MYB | 2 |
| ENSG00000121073 | SLC35B1 | 2 |
| ENSG00000123009 | NME2P1 | 2 |
| ENSG00000123268 | ATF1 | 2 |
| ENSG00000124508 | BTN2A2 | 2 |
| ENSG00000125245 | GPR18 | 2 |
| ENSG00000128272 | ATF4 | 2 |
| ENSG00000130545 | CRB3 | 2 |
| ENSG00000131236 | CAP1 | 2 |
| ENSG00000133316 | WDR74 | 2 |
| ENSG00000134160 | TRPM1 | 2 |
| ENSG00000134255 | CEPT1 | 2 |
| ENSG00000135048 | TMEM2 | 2 |
| ENSG00000135776 | ABCB10 | 2 |
| ENSG00000136156 | ITM2B | 2 |
| ENSG00000136875 | PRPF4 | 2 |
| ENSG00000138073 | PREB | 2 |
| ENSG00000139132 | FGD4 | 2 |

Supplementary table 6. (continued)

| Ensemble ID | Gene Name | Cluster |
|---|---|---|
| ENSG00000139644 | TMBIM6 | 2 |
| ENSG00000140090 | SLC24A4 | 2 |
| ENSG00000141258 | SGSM2 | 2 |
| ENSG00000144895 | EIF2A | 2 |
| ENSG00000149256 | TENM4 | 2 |
| ENSG00000151117 | TMEM86A | 2 |
| ENSG00000153048 | CARHSP1 | 2 |
| ENSG00000154277 | UCHL1 | 2 |
| ENSG00000154928 | EPHB1 | 2 |
| ENSG00000155066 | PROM2 | 2 |
| ENSG00000156261 | CCT8 | 2 |
| ENSG00000158301 | GPRASP2 | 2 |
| ENSG00000159352 | PSMD4 | 2 |
| ENSG00000159388 | BTG2 | 2 |
| ENSG00000160683 | CXCR5 | 2 |
| ENSG00000160752 | FDPS | 2 |
| ENSG00000160856 | FCRL3 | 2 |
| ENSG00000162482 | AKR7A3 | 2 |
| ENSG00000162733 | DDR2 | 2 |
| ENSG00000164099 | PRSS12 | 2 |
| ENSG00000164330 | EBF1 | 2 |
| ENSG00000164331 | ANKRA2 | 2 |
| ENSG00000164949 | GEM | 2 |
| ENSG00000165125 | TRPV6 | 2 |
| ENSG00000166295 | ANAPC16 | 2 |
| ENSG00000166747 | AP1G1 | 2 |
| ENSG00000167261 | DPEP2 | 2 |
| ENSG00000167508 | MVD | 2 |
| ENSG00000167515 | TRAPPC2L | 2 |
| ENSG00000167618 | LAIR2 | 2 |
| ENSG00000168159 | RNF187 | 2 |
| ENSG00000168389 | MFSD2A | 2 |
| ENSG00000170962 | PDGFD | 2 |
| ENSG00000171160 | MORN4 | 2 |
| ENSG00000171786 | NHLH1 | 2 |
| ENSG00000172932 | ANKRD13D | 2 |
| ENSG00000174282 | ZBTB4 | 2 |
| ENSG00000176155 | CCDC57 | 2 |
| ENSG00000177710 | SLC35G5 | 2 |
| ENSG00000178162 | FAR2P2 | 2 |
| ENSG00000178996 | SNX18 | 2 |
| ENSG00000181085 | MAPK15 | 2 |
| ENSG00000183092 | BEGAIN | 2 |
| ENSG00000183598 | HIST2H3D | 2 |
| ENSG00000184083 | FAM120C | 2 |

Supplementary table 6. (continued)

| Ensemble ID | Gene Name | Cluster |
|---|---|---|
| ENSG00000185404 | SP140L | 2 |
| ENSG00000185591 | SP1 | 2 |
| ENSG00000186642 | PDE2A | 2 |
| ENSG00000188004 | C1orf204 | 2 |
| ENSG00000188404 | SELL | 2 |
| ENSG00000188677 | PARVB | 2 |
| ENSG00000196455 | PIK3R4 | 2 |
| ENSG00000197081 | IGF2R | 2 |
| ENSG00000197558 | SSPO | 2 |
| ENSG00000203760 | CENPW | 2 |
| ENSG00000203799 | CCDC162P | 2 |
| ENSG00000203875 | SNHG5 | 2 |
| ENSG00000205853 | RFPL3S | 2 |
| ENSG00000211584 | SLC48A1 | 2 |
| ENSG00000211648 | IGLV1-47 | 2 |
| ENSG00000211668 | IGLV2-11 | 2 |
| ENSG00000211945 | IGHV1-18 | 2 |
| ENSG00000213015 | ZNF580 | 2 |
| ENSG00000213024 | NUP62 | 2 |
| ENSG00000213280 | | 2 |
| ENSG00000213714 | FAM209B | 2 |
| ENSG00000213983 | AP1G2 | 2 |
| ENSG00000214102 | WEE2 | 2 |
| ENSG00000214203 | RPS4XP1 | 2 |
| ENSG00000215493 | | 2 |
| ENSG00000219102 | HNRNPA3P12 | 2 |
| ENSG00000221955 | SLC12A8 | 2 |
| ENSG00000224578 | HNRNPA1P48 | 2 |
| ENSG00000225331 | | 2 |
| ENSG00000225523 | IGKV6D-21 | 2 |
| ENSG00000225569 | CCT4P2 | 2 |
| ENSG00000225695 | HNRNPA1P35 | 2 |
| ENSG00000226002 | | 2 |
| ENSG00000227492 | | 2 |
| ENSG00000228201 | | 2 |
| ENSG00000228403 | | 2 |
| ENSG00000229162 | | 2 |
| ENSG00000229897 | SEPT7P7 | 2 |
| ENSG00000229914 | | 2 |
| ENSG00000230869 | CTGLF10P | 2 |
| ENSG00000231128 | | 2 |
| ENSG00000231584 | FAHD2CP | 2 |
| ENSG00000233254 | RPL21P134 | 2 |
| ENSG00000233503 | HNRNPLP1 | 2 |
| ENSG00000234176 | HSPA8P1 | 2 |

Supplementary table 6. (continued)

| Ensemble ID | Gene Name | Cluster |
|---|---|---|
| ENSG00000234369 | TATDN1P1 | 2 |
| ENSG00000235688 | | 2 |
| ENSG00000236104 | ZBTB22 | 2 |
| ENSG00000236452 | | 2 |
| ENSG00000237307 | SRRM1P3 | 2 |
| ENSG00000237846 | | 2 |
| ENSG00000240376 | | 2 |
| ENSG00000240409 | MTATP8P1 | 2 |
| ENSG00000240652 | | 2 |
| ENSG00000240898 | | 2 |
| ENSG00000242076 | IGKV1-33 | 2 |
| ENSG00000243710 | CFAP57 | 2 |
| ENSG00000244021 | | 2 |
| ENSG00000244052 | RPL5P24 | 2 |
| ENSG00000244671 | RN7SL280P | 2 |
| ENSG00000244693 | CTAGE8 | 2 |
| ENSG00000247134 | | 2 |
| ENSG00000248697 | TOX4P1 | 2 |
| ENSG00000248795 | | 2 |
| ENSG00000250474 | WBP1LP2 | 2 |
| ENSG00000253047 | | 2 |
| ENSG00000253159 | PCDHGA12 | 2 |
| ENSG00000253910 | PCDHGB2 | 2 |
| ENSG00000254056 | IGHV3-71 | 2 |
| ENSG00000254470 | AP5B1 | 2 |
| ENSG00000254709 | IGLL5 | 2 |
| ENSG00000255318 | | 2 |
| ENSG00000258210 | | 2 |
| ENSG00000259321 | | 2 |
| ENSG00000259479 | SORD2P | 2 |
| ENSG00000259932 | | 2 |
| ENSG00000260018 | | 2 |
| ENSG00000261586 | | 2 |
| ENSG00000261812 | TUBB8P7 | 2 |
| ENSG00000262222 | | 2 |
| ENSG00000262434 | | 2 |
| ENSG00000264659 | | 2 |
| ENSG00000264660 | | 2 |
| ENSG00000267122 | | 2 |
| ENSG00000267784 | | 2 |
| ENSG00000268027 | | 2 |
| ENSG00000268056 | | 2 |
| ENSG00000268070 | | 2 |
| ENSG00000269102 | | 2 |
| ENSG00000269153 | LYPLA2P2 | 2 |

Supplementary table 6. (continued)

| Ensemble ID | Gene Name | Cluster |
|---|---|---|
| ENSG00000270264 | NDUFB8P2 | 2 |
| ENSG00000270558 | | 2 |
| ENSG00000270728 | | 2 |
| ENSG00000271321 | CTAGE6 | 2 |
| ENSG00000272745 | | 2 |
| ENSG00000274620 | MIR378J | 2 |
| ENSG00000278231 | | 2 |
| ENSG00000280123 | | 2 |
| ENSG00000280832 | ST3GAL4-AS1 | 2 |
| ENSG00000001629 | ANKIB1 | 3 |
| ENSG00000006459 | KDM7A | 3 |
| ENSG00000006576 | PHTF2 | 3 |
| ENSG00000015171 | ZMYND11 | 3 |
| ENSG00000015676 | NUDCD3 | 3 |
| ENSG00000021776 | AQR | 3 |
| ENSG00000029363 | BCLAF1 | 3 |
| ENSG00000033867 | SLC4A7 | 3 |
| ENSG00000034713 | GABARAPL2 | 3 |
| ENSG00000048028 | USP28 | 3 |
| ENSG00000057608 | GDI2 | 3 |
| ENSG00000058063 | ATP11B | 3 |
| ENSG00000064419 | TNPO3 | 3 |
| ENSG00000065809 | FAM107B | 3 |
| ENSG00000066294 | CD84 | 3 |
| ENSG00000068305 | MEF2A | 3 |
| ENSG00000069966 | GNB5 | 3 |
| ENSG00000072832 | CRMP1 | 3 |
| ENSG00000074603 | DPP8 | 3 |
| ENSG00000075151 | EIF4G3 | 3 |
| ENSG00000077232 | DNAJC10 | 3 |
| ENSG00000081237 | PTPRC | 3 |
| ENSG00000083857 | FAT1 | 3 |
| ENSG00000086062 | B4GALT1 | 3 |
| ENSG00000086570 | FAT2 | 3 |
| ENSG00000086589 | RBM22 | 3 |
| ENSG00000087448 | KLHL42 | 3 |
| ENSG00000088882 | CPXM1 | 3 |
| ENSG00000090776 | EFNB1 | 3 |
| ENSG00000092068 | SLC7A8 | 3 |
| ENSG00000095015 | MAP3K1 | 3 |
| ENSG00000101421 | CHMP4B | 3 |
| ENSG00000101444 | AHCY | 3 |
| ENSG00000104643 | MTMR9 | 3 |
| ENSG00000105483 | CARD8 | 3 |
| ENSG00000105656 | ELL | 3 |

Supplementary table 6. (continued)

| Ensemble ID | Gene Name | Cluster |
|---|---|---|
| ENSG00000105856 | HBP1 | 3 |
| ENSG00000105926 | MPP6 | 3 |
| ENSG00000106052 | TAX1BP1 | 3 |
| ENSG00000106069 | CHN2 | 3 |
| ENSG00000106538 | RARRES2 | 3 |
| ENSG00000107863 | ARHGAP21 | 3 |
| ENSG00000108219 | TSPAN14 | 3 |
| ENSG00000108587 | GOSR1 | 3 |
| ENSG00000109436 | TBC1D9 | 3 |
| ENSG00000110315 | RNF141 | 3 |
| ENSG00000110330 | BIRC2 | 3 |
| ENSG00000110777 | POU2AF1 | 3 |
| ENSG00000111837 | MAK | 3 |
| ENSG00000111913 | FAM65B | 3 |
| ENSG00000113441 | LNPEP | 3 |
| ENSG00000113532 | ST8SIA4 | 3 |
| ENSG00000114439 | BBX | 3 |
| ENSG00000115020 | PIKFYVE | 3 |
| ENSG00000115170 | ACVR1 | 3 |
| ENSG00000115956 | PLEK | 3 |
| ENSG00000116191 | RALGPS2 | 3 |
| ENSG00000116406 | EDEM3 | 3 |
| ENSG00000116514 | RNF19B | 3 |
| ENSG00000117009 | KMO | 3 |
| ENSG00000118307 | CASC1 | 3 |
| ENSG00000118308 | LRMP | 3 |
| ENSG00000118412 | CASP8AP2 | 3 |
| ENSG00000118898 | PPL | 3 |
| ENSG00000119285 | HEATR1 | 3 |
| ENSG00000119729 | RHOQ | 3 |
| ENSG00000119862 | LGALSL | 3 |
| ENSG00000120063 | GNA13 | 3 |
| ENSG00000120963 | ZNF706 | 3 |
| ENSG00000121101 | TEX14 | 3 |
| ENSG00000122786 | CALD1 | 3 |
| ENSG00000126216 | TUBGCP3 | 3 |
| ENSG00000127152 | BCL11B | 3 |
| ENSG00000127947 | PTPN12 | 3 |
| ENSG00000128203 | ASPHD2 | 3 |
| ENSG00000130175 | PRKCSH | 3 |
| ENSG00000130382 | MLLT1 | 3 |
| ENSG00000131127 | ZNF141 | 3 |
| ENSG00000131747 | TOP2A | 3 |
| ENSG00000132128 | LRRC41 | 3 |
| ENSG00000133789 | SWAP70 | 3 |

Supplementary table 6. (continued)

| Ensemble ID | Gene Name | Cluster |
|---|---|---|
| ENSG00000134061 | CD180 | 3 |
| ENSG00000134278 | SPIRE1 | 3 |
| ENSG00000135090 | TAOK3 | 3 |
| ENSG00000135316 | SYNCRIP | 3 |
| ENSG00000135519 | KCNH3 | 3 |
| ENSG00000136040 | PLXNC1 | 3 |
| ENSG00000136051 | KIAA1033 | 3 |
| ENSG00000136367 | ZFHX2 | 3 |
| ENSG00000137502 | RAB30 | 3 |
| ENSG00000138101 | DTNB | 3 |
| ENSG00000138593 | SECISBP2L | 3 |
| ENSG00000140350 | ANP32A | 3 |
| ENSG00000140391 | TSPAN3 | 3 |
| ENSG00000140396 | NCOA2 | 3 |
| ENSG00000140406 | MESDC1 | 3 |
| ENSG00000140525 | FANCI | 3 |
| ENSG00000141522 | ARHGDIA | 3 |
| ENSG00000142197 | DOPEY2 | 3 |
| ENSG00000142330 | CAPN10 | 3 |
| ENSG00000143297 | FCRL5 | 3 |
| ENSG00000143878 | RHOB | 3 |
| ENSG00000143924 | EML4 | 3 |
| ENSG00000144118 | RALB | 3 |
| ENSG00000144711 | IQSEC1 | 3 |
| ENSG00000145246 | ATP10D | 3 |
| ENSG00000145725 | PPIP5K2 | 3 |
| ENSG00000146757 | ZNF92 | 3 |
| ENSG00000149483 | TMEM138 | 3 |
| ENSG00000153922 | CHD1 | 3 |
| ENSG00000155657 | TTN | 3 |
| ENSG00000156414 | TDRD9 | 3 |
| ENSG00000156738 | MS4A1 | 3 |
| ENSG00000158865 | SLC5A11 | 3 |
| ENSG00000159208 | CIART | 3 |
| ENSG00000159231 | CBR3 | 3 |
| ENSG00000159648 | TEPP | 3 |
| ENSG00000160282 | FTCD | 3 |
| ENSG00000160294 | MCM3AP | 3 |
| ENSG00000160310 | PRMT2 | 3 |
| ENSG00000162105 | SHANK2 | 3 |
| ENSG00000162512 | SDC3 | 3 |
| ENSG00000162772 | ATF3 | 3 |
| ENSG00000162980 | ARL5A | 3 |
| ENSG00000163126 | ANKRD23 | 3 |
| ENSG00000163349 | HIPK1 | 3 |

Supplementary table 6. (continued)

| Ensemble ID | Gene Name | Cluster |
|---|---|---|
| ENSG00000163635 | ATXN7 | 3 |
| ENSG00000163939 | PBRM1 | 3 |
| ENSG00000164344 | KLKB1 | 3 |
| ENSG00000164530 | PI16 | 3 |
| ENSG00000164638 | SLC29A4 | 3 |
| ENSG00000164754 | RAD21 | 3 |
| ENSG00000164855 | TMEM184A | 3 |
| ENSG00000164941 | INTS8 | 3 |
| ENSG00000165025 | SYK | 3 |
| ENSG00000165794 | SLC39A2 | 3 |
| ENSG00000166340 | TPP1 | 3 |
| ENSG00000166532 | RIMKLB | 3 |
| ENSG00000166783 | KIAA0430 | 3 |
| ENSG00000167103 | PIP5KL1 | 3 |
| ENSG00000167306 | MYO5B | 3 |
| ENSG00000168214 | RBPJ | 3 |
| ENSG00000168438 | CDC40 | 3 |
| ENSG00000169045 | HNRNPH1 | 3 |
| ENSG00000169504 | CLIC4 | 3 |
| ENSG00000169508 | GPR183 | 3 |
| ENSG00000169554 | ZEB2 | 3 |
| ENSG00000169967 | MAP3K2 | 3 |
| ENSG00000170128 | GPR25 | 3 |
| ENSG00000170160 | CCDC144A | 3 |
| ENSG00000170471 | RALGAPB | 3 |
| ENSG00000170638 | TRABD | 3 |
| ENSG00000171316 | CHD7 | 3 |
| ENSG00000171812 | COL8A2 | 3 |
| ENSG00000172340 | SUCLG2 | 3 |
| ENSG00000173821 | RNF213 | 3 |
| ENSG00000173825 | TIGD3 | 3 |
| ENSG00000174197 | MGA | 3 |
| ENSG00000174527 | MYO1H | 3 |
| ENSG00000175514 | GPR152 | 3 |
| ENSG00000175893 | ZDHHC21 | 3 |
| ENSG00000176623 | RMDN1 | 3 |
| ENSG00000176953 | NFATC2IP | 3 |
| ENSG00000177272 | KCNA3 | 3 |
| ENSG00000178295 | GEN1 | 3 |
| ENSG00000178971 | CTC1 | 3 |
| ENSG00000179456 | ZBTB18 | 3 |
| ENSG00000180398 | MCFD2 | 3 |
| ENSG00000181090 | EHMT1 | 3 |
| ENSG00000182180 | MRPS16 | 3 |
| ENSG00000183022 | TPM3P8 | 3 |

Supplementary table 6. (continued)

| Ensemble ID | Gene Name | Cluster |
|---|---|---|
| ENSG00000183615 | FAM167B | 3 |
| ENSG00000184445 | KNTC1 | 3 |
| ENSG00000185436 | IFNLR1 | 3 |
| ENSG00000185946 | RNPC3 | 3 |
| ENSG00000186432 | KPNA4 | 3 |
| ENSG00000188177 | ZC3H6 | 3 |
| ENSG00000188873 | RPL10AP2 | 3 |
| ENSG00000188906 | LRRK2 | 3 |
| ENSG00000196628 | TCF4 | 3 |
| ENSG00000196664 | TLR7 | 3 |
| ENSG00000197321 | SVIL | 3 |
| ENSG00000197629 | MPEG1 | 3 |
| ENSG00000197943 | PLCG2 | 3 |
| ENSG00000198156 | NPIPB6 | 3 |
| ENSG00000198399 | ITSN2 | 3 |
| ENSG00000198590 | C3orf35 | 3 |
| ENSG00000198743 | SLC5A3 | 3 |
| ENSG00000198901 | PRC1 | 3 |
| ENSG00000203279 | | 3 |
| ENSG00000205918 | PDPK2P | 3 |
| ENSG00000211678 | IGLJ3 | 3 |
| ENSG00000211897 | IGHG3 | 3 |
| ENSG00000213051 | RPL5P5 | 3 |
| ENSG00000213057 | C1orf220 | 3 |
| ENSG00000213731 | RAB5CP1 | 3 |
| ENSG00000213859 | KCTD11 | 3 |
| ENSG00000214013 | GANC | 3 |
| ENSG00000214367 | HAUS3 | 3 |
| ENSG00000215347 | SLC25A5P1 | 3 |
| ENSG00000215529 | EFCAB8 | 3 |
| ENSG00000218418 | | 3 |
| ENSG00000223356 | | 3 |
| ENSG00000223984 | HNRNPRP1 | 3 |
| ENSG00000224029 | | 3 |
| ENSG00000225447 | RPS15AP10 | 3 |
| ENSG00000226833 | | 3 |
| ENSG00000227077 | | 3 |
| ENSG00000228050 | TOP3BP1 | 3 |
| ENSG00000228285 | LYPLA2P1 | 3 |
| ENSG00000229196 | | 3 |
| ENSG00000229204 | PTGES3P3 | 3 |
| ENSG00000229604 | MTATP8P2 | 3 |
| ENSG00000230592 | RPSAP8 | 3 |
| ENSG00000230756 | RHOQP3 | 3 |
| ENSG00000231043 | | 3 |

Supplementary table 6. (continued)

| Ensemble ID | Gene Name | Cluster |
|---|---|---|
| ENSG00000231064 | | 3 |
| ENSG00000231466 | | 3 |
| ENSG00000233155 | HMGA1P8 | 3 |
| ENSG00000233328 | PFN1P1 | 3 |
| ENSG00000233837 | EIF3LP2 | 3 |
| ENSG00000234005 | GAPDHP22 | 3 |
| ENSG00000235238 | SUMO2P1 | 3 |
| ENSG00000235472 | EIF4A1P7 | 3 |
| ENSG00000235587 | GAPDHP65 | 3 |
| ENSG00000236953 | ZDHHC20-IT1 | 3 |
| ENSG00000237286 | | 3 |
| ENSG00000237296 | SMG1P1 | 3 |
| ENSG00000238083 | LRRC37A2 | 3 |
| ENSG00000239911 | PRKAG2-AS1 | 3 |
| ENSG00000240563 | L1TD1 | 3 |
| ENSG00000241255 | | 3 |
| ENSG00000243431 | RPL5P30 | 3 |
| ENSG00000243716 | NPIPB5 | 3 |
| ENSG00000244617 | ASPRV1 | 3 |
| ENSG00000245532 | NEAT1 | 3 |
| ENSG00000246560 | | 3 |
| ENSG00000249244 | | 3 |
| ENSG00000249459 | ZNF286B | 3 |
| ENSG00000250506 | CDK3 | 3 |
| ENSG00000250848 | | 3 |
| ENSG00000251495 | PPIAP11 | 3 |
| ENSG00000253209 | IGHV3-65 | 3 |
| ENSG00000255220 | DDX18P5 | 3 |
| ENSG00000255224 | | 3 |
| ENSG00000258477 | PPIAP6 | 3 |
| ENSG00000258613 | RPL21P7 | 3 |
| ENSG00000258947 | TUBB3 | 3 |
| ENSG00000259144 | RANBP20P | 3 |
| ENSG00000259746 | HSPE1P3 | 3 |
| ENSG00000260265 | | 3 |
| ENSG00000260571 | BNIP3P5 | 3 |
| ENSG00000261399 | | 3 |
| ENSG00000261441 | | 3 |
| ENSG00000262691 | | 3 |
| ENSG00000263675 | MIR5581 | 3 |
| ENSG00000266993 | | 3 |
| ENSG00000268222 | EEF1A1P7 | 3 |
| ENSG00000269506 | | 3 |
| ENSG00000271360 | | 3 |
| ENSG00000272977 | | 3 |

Supplementary table 6. (continued)

| Ensemble ID | Gene Name | Cluster |
|---|---|---|
| ENSG00000277363 | SRCIN1 | 3 |
| ENSG00000277692 | | 3 |
| ENSG00000278917 | | 3 |
| ENSG00000280353 | | 3 |
| ENSG00000281207 | SLFNL1-AS1 | 3 |

**Supplementary table 7:** Differentially expressed genes (387) between JIA patients without uveitis and JIA patients with uveitis.

WEBLINK: https://www.frontiersin.org/articles/10.3389/fimmu.2020.02170/full#supplementary-material

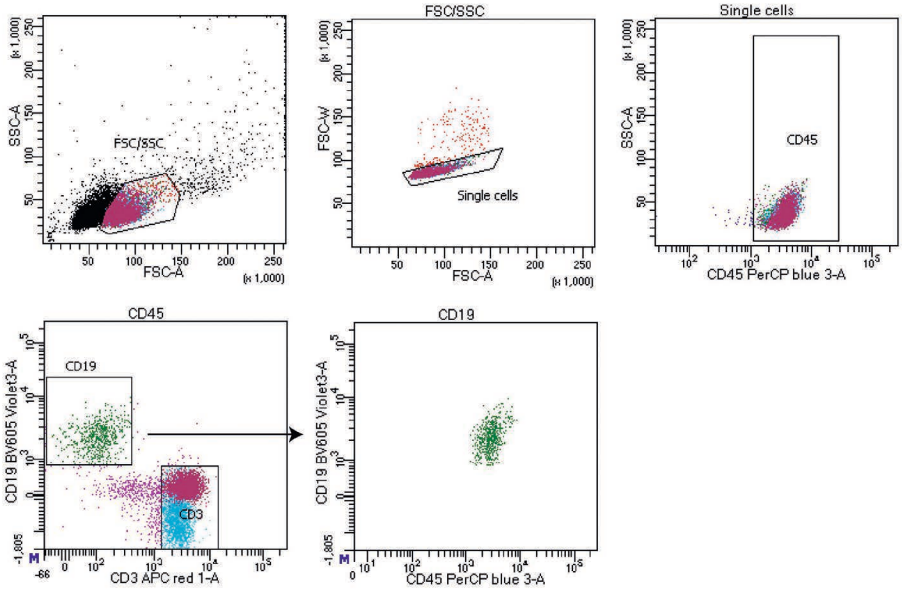
**Supplementary table 8:** Differentially expressed genes (878) at nominal significance between JIA patients without uveitis, JIA patients with uveitis 1 and JIA patients with uveitis 2.

WEBLINK: https://www.frontiersin.org/articles/10.3389/fimmu.2020.02170/full#supplementary-material

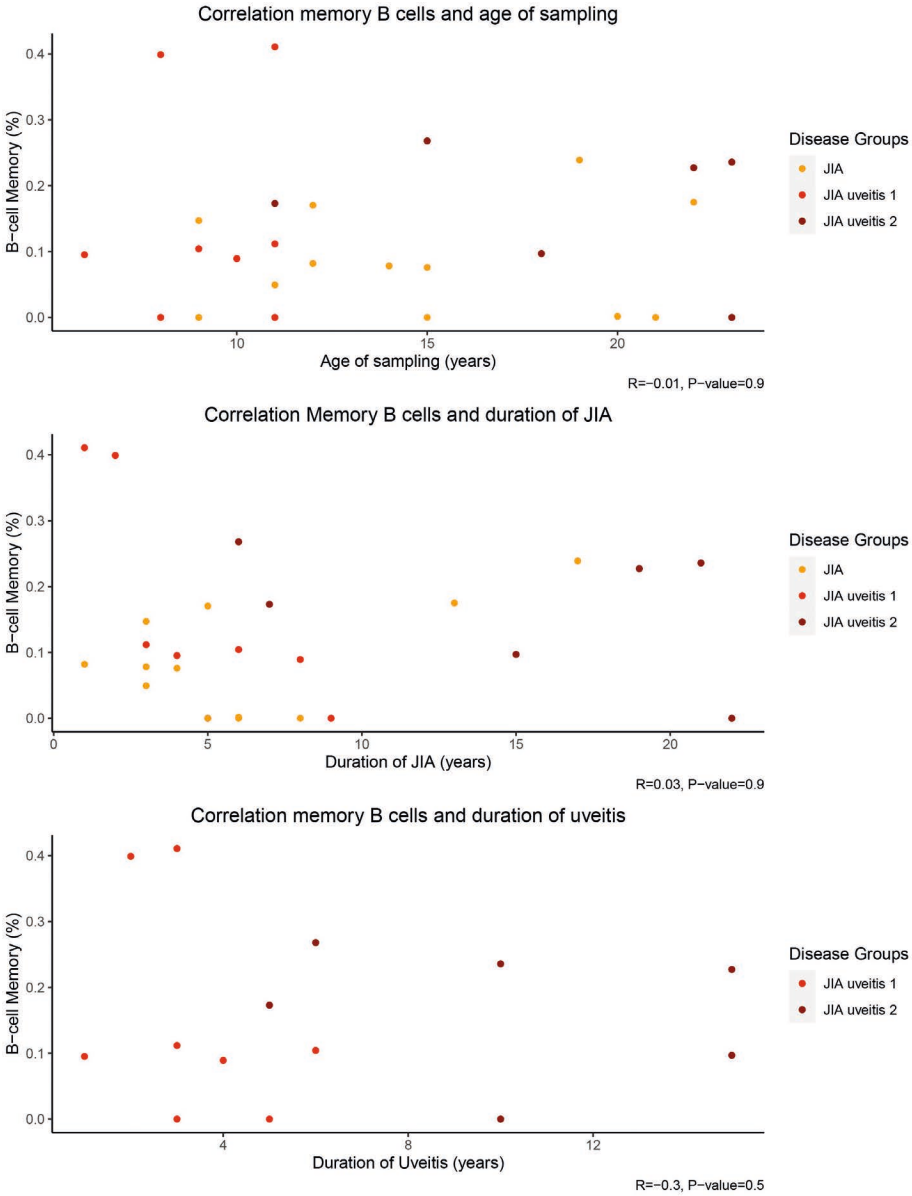
**Supplementary table 9:** Differentially expressed genes (485) at false discovery rate of 5% between JIA patients with uveitis 1 and JIA patients with uveitis 2.

WEBLINK: https://www.frontiersin.org/articles/10.3389/fimmu.2020.02170/full#supplementary-material

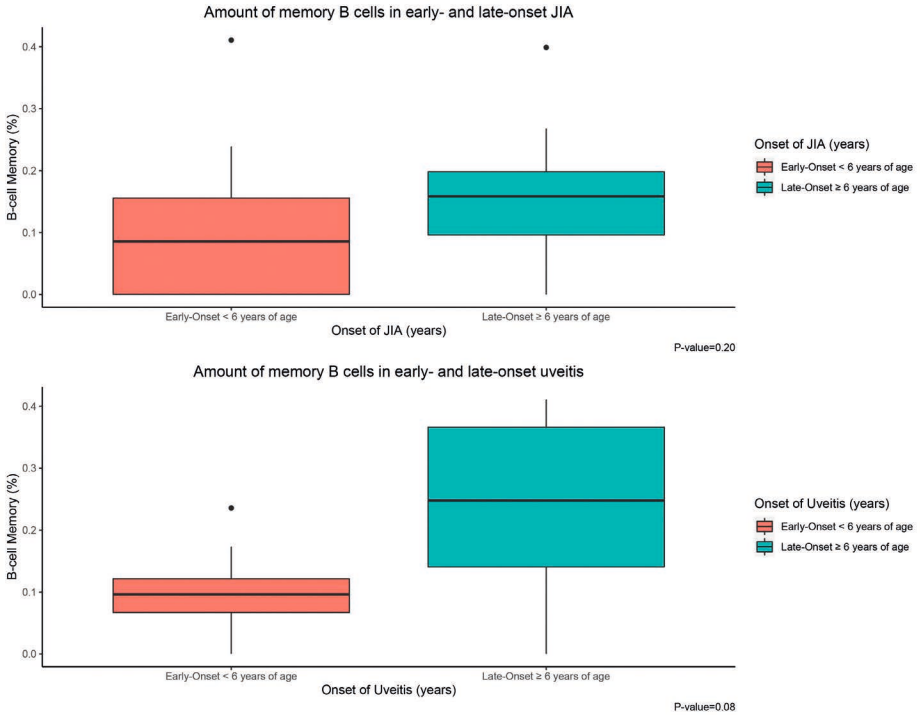
## Supplementary figures



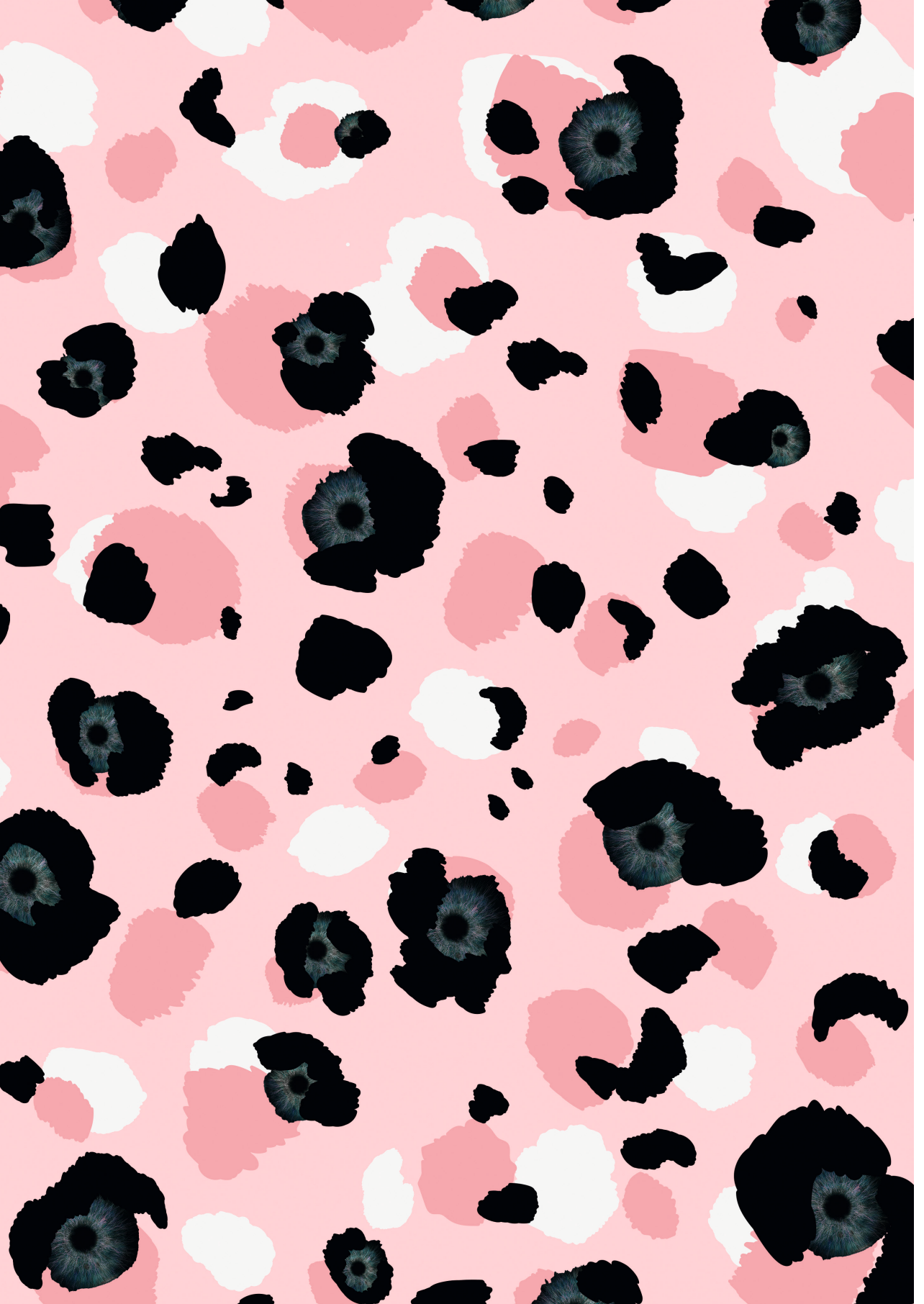

**Supplementary figure 1.** Gating strategy used for FACS sorting of peripheral CD45+CD3-CD19+ B cells.




**Supplementary figure 2.** No correlation between memory B cells and age or disease duration.




**Supplementary figure 3**. Amount of memory B cells in early and late-onset of disease.



# CHAPTER 7

## Next-Generation HLA sequence analysis uncovers shared risk alleles between clinically distinct forms of childhood uveitis

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## ABSTRACT

**Purpose:** Classical alleles of the *human leukocyte antigen* (*HLA*) complex have been linked to specific entities of pediatric noninfectious uveitis, yet genetic predisposition encoded by the HLA super-locus across the patient population remains understudied.

**Methods**: We performed next-generation full-length sequencing of *HLA-A, HLA-B, HLA-C*, *HLA-DPB1, HLA-DQB1,* and *HLA-DRB1* in 280 cases. Dense genotype data from 499 Dutch controls from *Genome of the Netherlands* were imputed using an HLA-specific reference panel (n=5,225 samples from European ancestry). Cases and controls were compared using logistic regression models adjusting for sex.

**Results** In total, 179 common and rare alleles were detected. Considering all cases and controls, *HLA-DQB1*04:02* and *HLA-DRB1*08:01* were identified as the principal *HLA* association, which was mainly driven by 92 cases with *juvenile idiopathic arthritis-associated uveitis* (JIA-U). The *HLA-DQB1*04:02-HLA-DRB1*08:01* haplotype was also the primary association for the phenotypically similar *idiopathic chronic anterior uveitis* without arthritis (CAU). Also, *HLA-DQB1*05:03* was an independent risk allele for CAU, but not in JIA-U. Analysis of 185 cases with other forms of uveitis revealed HLA-wide associations ($P<2.79\times10^{-4}$) for *HLA-DRB1*01:02, HLA-DRB1*04:03,* and *HLA-DQB1*05:03*, which could be primarily attributed to cases with panuveitis. Finally, amino acid substitution modeling revealed that aspartic acid at position 57 that distinguishes the risk allele *HLA-DQB1*05:03* (for CAU and panuveitis) from nonrisk alleles, significantly increased the binding capacity of naturally presented ligands to HLA-DQ.

**Conclusions:** These results uncovered novel shared *HLA* associations among clinically distinct phenotypes of pediatric uveitis and highlight genetic predisposition affecting the antigen presentation pathway.

## INTRODUCTION

Noninfectious pediatric uveitis poses a significant burden on the health and quality of life of affected children, because of its high risk of visual impairment and its lifelong dependency on specialized care.[1,2] Despite the variety of clinical phenotypes, little is understood of the underlying disease mechanisms that render some children prone to develop uveitis. Consequently, due to lack of understanding their causes, at least half of cases are considered idiopathic and therefore left to be classified according to the affected areas (i.e. anterior uveitis describes cases with a predominant involvement of the anterior segment of the eye).[3] Regardless, immune-mediated etiology is strongly suspected because pediatric uveitis occurs often in cases suffering from autoimmune diseases (e.g., rheumatic disease). In fact, the largest category (12%-30%) of pediatric uveitis is cases suffering from anterior uveitis associated with preexisting juvenile idiopathic arthritis (JIA).[4] In addition, immunosuppressive therapy regimes are effective in dampening eye inflammation and further support that derailed immunity is an important contributor of disease.[5-8]

Genetic studies that strongly linked several alleles of the family of *human leukocyte antigen* (*HLA*) genes on chromosome 6 to noninfectious uveitis add to the body of evidence.[9-15] HLA molecules are critically involved in adaptive immunity by presenting protein fragments sampled from the cellular (class I HLA molecules) and extracellular (class II HLA molecules) environment to surveilling T and natural killer cells to orchestrate antigen-specific immunity.[16] Genetic studies of pediatric uveitis have mostly been conducted in JIA-U cases of Western European ancestry, which mapped the primary genetic risk for uveitis in JIA to amino acid positions 10 to 12 encoded by the HLA–DRB1 gene.[14] This uveitis motif exhibited a disease risk in females which was independent from the known genetic association of JIA with other amino acid positions in *HLA-DRB1*.[14] This example demonstrated that different polymorphisms in the same gene may confer independent disease risks and supported that uveitis is a distinct trait of the pediatric population.[14,15] Other HLA typing studies have been conducted in disease-specific contexts, such as sequence specific typing association studies in small cohorts of cases with tubulointerstitial nephritis and uveitis (TINU) syndrome.[17-22] At present, *HLA* typing studies across a representative sample of pediatric uveitis population are sparse. Here, we performed allelic resolution next-generation sequencing across all classical class I and class II *HLA* alleles to refine

*HLA* associations and amino acids polymorphisms at the population level and stratify *HLA* haplotypes according to anatomical categories and specific entities.

## METHODS

### Patient and material collection

The study was approved by the Medical Ethical Research Committee of the University Medical Center Utrecht in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients ≥ 18 years, from both parents and patients in cases between 12 to 18 years of age, and from parents in cases < 12 years old. Peripheral blood samples (EDTA tubes) were obtained from patients with juvenile idiopathic arthritis-associated uveitis (JIA-U, n=92), idiopathic chronic anterior uveitis (i.e., no JIA) (CAU, n=50), (*HLA-B27*-positive) acute anterior uveitis (n=18), intermediate uveitis (IU, n=39), and panuveitis (PAN, n=81). The diagnosis of uveitis was established by a trained uveitis specialist according to the SUN criteria.[23] All cases were recruited at the University Medical Center Utrecht, the Netherlands, a tertiary referral center. The study only considered patients with a diagnosis of noninfectious uveitis before the age of 18 years. Juvenile idiopathic arthritis was diagnosed according to the criteria of the *International League of Associations for Rheumatology* or by former criteria (e.g., *European League Against Rheumatism*).[24,25] Patients with JIA were screened by an ophthalmologist according to the guidelines of the Academy of Pediatrics.[26] Patients with 1+ cells or more in the anterior chamber and treated with at least topical steroids during ophthalmologic examinations, were diagnosed with JIA-U. The diagnosis for (probable or possible) TINU syndrome was made by an ophthalmologist specialized in pediatric uveitis and a pediatric nephrologist based on clinical criteria.[17]

### HLA typing by High-Resolution Next-Generation Sequencing

DNA was isolated from 300µL of peripheral blood (EDTA tubes) using the MagNa Pure Compact (Roche Diagnostics, the Netherlands). From genomic DNA, coding sequences of the *HLA* region were enriched by PCR using multiplexed HLA amplification by NGSgo-MX6-1 PCR (GenDx, Utrecht, The Netherlands). Next-generation sequencing was performed on all samples using the Illumina sequencing system. The DNA fragment were analyzed with the *NGSEngine* software package version 2.14.0 (GenDx, Utrecht, the Netherlands) using the IPD-IMGT/HLA database version 3.36.0. After inspection of the full-length sequence, the genotype data for 179 distinct *HLA* alleles were reduced to second field nomenclature to reflect the

unambiguous phenotype. The corresponding amino acid sequences for each of the 179 alleles was obtained from the *Immuno Polymorphism Database* (IPD) and the international ImMunoGeneTics (IMGT)/HLA release 3.29[27] (**Supplementary table 1A-F**). A total of 293 amino acid positions were considered polymorphic and used for fine-mapping of amino acid associations (**Supplementary table 6**).

**Reference cohort**

Genotype data from 499 unrelated and unaffected Dutch subjects from the Genome of the Netherlands (GoNL) were used as population controls.[28] The *GoNL project* is a human genetic project based on a representative sample of Dutch citizens from all provinces in the Netherlands, which captures the genetic variation in the Dutch population (for more information see http://www.nlgenome.nl). Next, we used BEAGLE package to prephase the GoNL single nucleotide polymorphism (SNP) data for imputation. In other words, we estimated the haplotypes for each individual GoNL sample for efficient imputation of ungenotyped variants based on linkage disequilibrium.[29] We imputed classical *HLA* alleles using SNP data and analysis with the SNP2HLA pipeline, which given the strong linkage disequilibrium in the *MHC* region, can be used to tag the majority of *HLA* alleles. The SNP2HLA pipeline uses a reference panel assembled through HLA typing of 5225 European-ancestry individuals collected by the Type 1 Diabetes Genetics Consortium to ascertain accurate HLA imputation.[30]

**HLA association analysis**

Sequence data were converted to dosage data by coding the presence or absence of each classical allele or encoded amino acid residue at indicated positions to 0,1, or 2. This generated three possible genotypes: homozygous (2) positive, heterozygous (1) positive, and homozygous negative (0) of the classical *HLA* allele/amino acid residue at a position. To visualize the ethnicity of the cases and controls, we used *Uniform Manifold Approximation and Projection* (UMAP)[31] based on the top 60 principal components calculated using the dosage data. For this analysis we also included HLA genotypes from populations of the 1000 Genomes Project (**Supplementary table 2**).[32,33] *HLA* association testing was performed using PLINK 2.0 using an additive logistic regression model with sex as a covariate.[34] To identify potential independent associations for *HLA* alleles and amino acid residues, we performed a series of association analyses adjusting for the primary association (i.e., conditional analysis), by adding each significantly associated allele as a covariate to the model. Our data

enabled multiple comparisons: 1) pediatric noninfectious uveitis versus controls, 2) JIA-U versus controls, 3) non-JIA-U versus controls, 4) CAU versus controls, 5) IU versus controls, and 6) PAN versus controls. A *P* value threshold of $2.79 \times 10^{-4}$ (*Bonferroni* correction; 0.05/179 detected *HLA* alleles) was used as a threshold for significance to prioritize reporting of *HLA* associations and termed this threshold "HLA-wide" significance.

**Amino acid substitution modeling**

To explore whether the presence of naturally occurring amino acids at position 57 of the different *HLA-DQB1*05* alleles affected the binding of peptide cargo to the HLA molecule, we extracted 157 ligands identified by mass spectrometry (from the HLA ligand atlas) from elutions of immunoprecipitations of *HLA-DQB1*05:01, HLA-DQB1*05:02*, and *HLA-DQB1*05:03*.[35] Replacement of position 57 with the amino acids at that position in the *HLA-DQB1*05* alleles was done in the *NetMHCIIpan* 4.0 server using the amino acid sequence of *HLA-DQB1*05* (UniProt accession: Q9GIK3) and the binding of each of the 157 predicted ligands using default settings.[36] Differences in the median binding score were calculated using a Wilcoxon signed rank test. We computed the peptide motif of the 157 peptide ligands using the artificial neural network model of *NNAlign-2.0*, which used the amino acid sequences and binding scores for each peptide for *HLA-DQB*05:03* as input (trained with a motif length of 9 and default settings for "MHC CLASS II ligands") to return a peptide core sequence alignment.[37] We also did this for a subset of the 157 peptides with a relatively stronger binding score to *HLA-DQB*05:03* (D57) compared to *DQB1:05:02* (V57), by computing the ratio of the binding scores (the log-transformed IC50 affinity scores from NetMHCIIpan 4.0) for all 157 peptides for those two alleles and extracting the top 50 peptides with the largest ratio.

## RESULTS

In total 280 cases with uveitis were genotyped of which 160 (57%) patients were diagnosed with anterior uveitis, 39 (14%) patients with intermediate uveitis, and 81 (29%) patients with panuveitis (**Table 1**). Summary of the HLA genotypes at four-digit resolution revealed 110 unique common alleles (≥1% detected in controls) and 69 rare alleles (<1% in controls) of which included 32 *HLA-A* alleles, 51 *HLA-B* alleles, 22 *HLA-C* alleles, 23 *HLA-DQB1* alleles, 13 *HLA-DPB1* alleles, and 38 *HLA-DRB1* alleles (**Supplementary table 3**). Uniform manifold approximation and projection

was used to visualize the ethnicity of the cases and controls (**Supplementary table 2**). As expected, cases and controls were predominantly mapped to the European population (**Figure 1**).

**Table 1.** Diagnosis of the 280 uveitis cases

| Diagnosis | Number of cases |
|---|---|
| Pediatric noninfectious uveitis | 280 |
| Anterior uveitis, No. (%) | 160 (57) |
| JIA associated uveitis, No. (%) | 92 (33) |
| Idiopathic chronic anterior uveitis, No. (%) | 50 (18) |
| Acute anterior uveitis, No. (%) [a] | 18 (6) |
| Non anterior uveitis, No. (%) | 120 (43) |
| Intermediate uveitis, No. (%) [b] | 39 (14) |
| Panuveitis, No. (%) [c] | 81 (29) |

[a] *HLA B27* positive associated uveitis (n=7), sarcoidosis (n=1), and TINU syndrome (n=2)
[b] Tubulointerstitial nephritis and uveitis syndrome (n=1)
[c] Tubulointerstitial nephritis and uveitis syndrome (n=15)

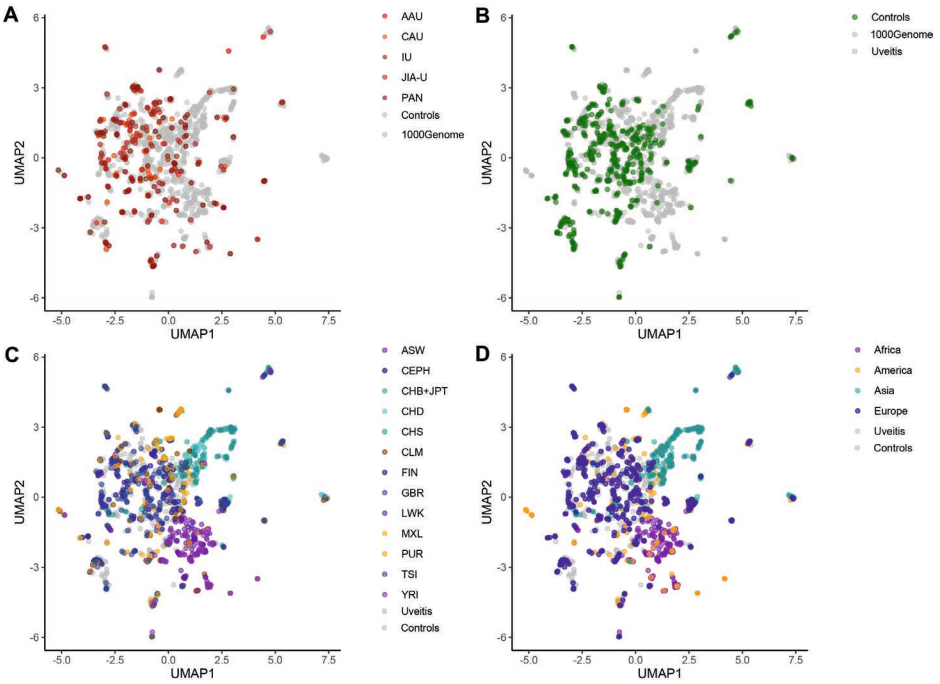


**Figure 1.** Uniform manifold approximation and projection of the HLA genotypes data.

Ancestry populations from the 1000genome dataset [32,33] (**Supplementary table 2**) and Dutch subjects from the *Genome of the Netherlands* was used as reference data to analyze ethnicity. The UMAP projection mapped cases and controls predominantly to the European population. **A)** Visualization of data from uveitis samples (n=280) used in this study. The different colors represent the five different forms of pediatric uveitis. **B)** Visualization of data from the control samples (n=499) from the *Genome of the Netherlands*. **C)** Visualization of data from the 1000genome per population code. **D)** Visualization of data from the 1000genome per super population. AAU, acute anterior uveitis; CAU, idiopathic chronic associated uveitis; JIA-U, juvenile idiopathic arthritis associated uveitis; IU, intermediate uveitis; PAN, panuveitis; African (LWK, Luhya in Kenya; YRI, Yoruba in Nigeria; ASW, Americans of African Ancestry in Southwest USA); European (CEPH , Utah residents with Northern/Western European ancestry; FIN, Finnish in Finland; GBR, British in England and Scotland; TSI, Tuscans in Italy); East Asian (CHB, Han Chinese in Beijing; CHS, Southern Han Chinese; JPT, Japanese in Tokyo, and CHD, Chinese in Metropolitan Denver CO, USA); and Mixed American (CLM, Colombian in Medellin, Colombia; MXL, Mexican in Los Angeles, California USA and PUR, Puerto Rican).

### *HLA-DRB1* and *HLA-DQB1* are risk alleles for pediatric uveitis

Next, we tested for *HLA* associations considering all uveitis cases (n=280). We identified seven associated *HLA* alleles and mapped the primary association to *HLA-DQB1*04:02* (odds ratio [OR] = 5.27, 95% confidence interval [95% CI] 3.04-9.12; $P = 2.98 \times 10^{-9}$), closely followed by *HLA-DRB1*08:01* (OR = 5.21 95% CI; 2.94-9.23; $P = 1.53 \times 10^{-8}$) (**Figure 2A**). The results from the association testing of the classical *HLA* alleles in all cases and controls are presented in **Supplementary table 3.** All of

the associated alleles were common (allele frequency in controls >1%). Since the haplotype *DRB1*08:01-DQB1*04:02* has been previously associated with *polyarticular rheumatoid factor-negative* JIA[38,39], we extracted the data from the 95 JIA cases and conducted association testing. For JIA-U versus controls, we ascertained the strong association to known JIA risk alleles *HLA-DRB1*08:01* (OR = 11.12, 95% CI 5.76-21.43; $P = 6.51 \times 10^{-13}$) and *HLA-DQB1*04:02* (OR = 10.11, 95% CI 5.32-19.22; $P = 1.69 \times 10^{-12}$) (**Figure 2B**). Next, we invested all independent *HLA* associations by using conditional analysis. To this end we adjusted for the strongest *HLA* association by adding the allele as a covariate to the regression model. Adjusting for *HLA-DRB1*08:01* mitigated the association for *HLA-DQB1*04:02* due to strong linkage disequilibrium (i.e., correlation [LD]) between these alleles (D'=0.97, $r^2$=0.86 in our data, **Table 2**). However, adjusting for *HLA-DRB1*08:01* revealed an independent association for *HLA-DRB1*11:04* (**Table 2**). Finally, after adjusting for all three *HLA* alleles (*DRB1*08:01-DQB1*04:02-HLA-DRB1*11:04*), no residual HLA-wide association signal was observed ($P > 2.79 \times 10^{-4}$).

Association testing in the 185 uveitis cases without JIA revealed a primary association for *HLA-DRB1*01:02* (OR = 5.58, 95% CI 2.70-11.55; $P = 3.60 \times 10^{-6}$) (**Figure 2C**). Adjusting for *HLA-DRB1*01:02* revealed an independent association for *HLA-DRB1*04:03 (*OR = 11.67, 95% CI 3.82-35.66; $P = 1.62 \times 10^{-5}$). Adjusting for both *DRB1*01:02* and *DRB1*04:03* revealed an additional independent association for *HLA-DQB1*04:02* (OR = 3.82, 95% CI 2.01-7.25; $P = 4.21 \times 10^{-5}$).

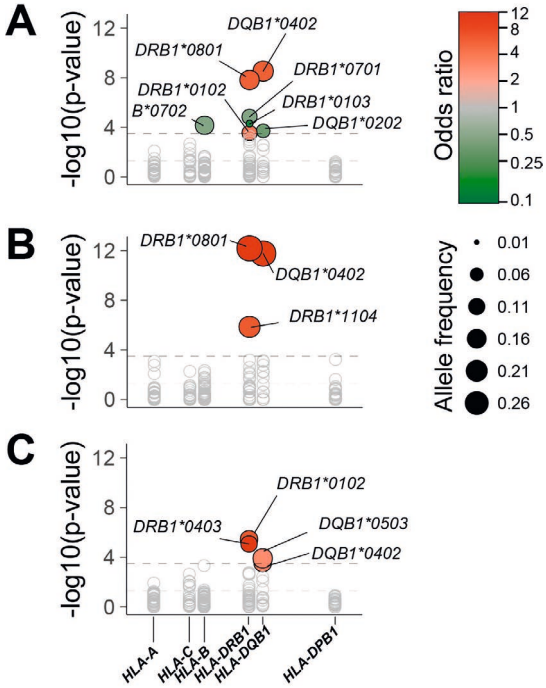


**Figure 2.** Association testing for *human leukocyte* alleles in all uveitis cases (n=280), JIA-uveitis (n=92), and uveitis without JIA-uveitis (n=185) versus controls (n=499).

**A)** Initial association testing for all uveitis cases revealed a primary association for *HLA-DQB1*04:02* (AF= 0.09, OR = 5.27, 95% CI 3.04-9.12; $P = 2.98 \times 10^{-9}$), closely followed by *HLA-DRB1*08:01* (AF=0.08, OR = 5.21 95% CI; 2.94-9.23; $P = 1.53 \times 10^{-8}$). **B)** Initial association testing for juvenile idiopathic associated uveitis cases (n=92) showed a primary association for *HLA-DRB1*08:01* (AF=0.16, OR = 11.12, 95% CI 5.76-21.43; $P = 6.51 \times 10^{-13}$) and *HLA-DQB1*04:02* (AF=0.16, OR = 10.11, 95% CI 5.32-19.22; $P = 1.69 \times 10^{-12}$) **C)** Initial association testing for uveitis cases without juvenile idiopathic associated uveitis (n=185) revealed a primary association for *HLA-DRB1*01:02* (AF=0.06. OR = 5.58, 95% CI 2.70-11.55; $P = 3.60 \times 10^{-6}$). All indicated *HLA* alleles are associated at HLA-wide significance ($P<2.79 \times 10^{-4}$, dashed dark gray line). The size and color of the dots represent the allele frequency and effect size (odds ratio), respectively. The gray dots represent the *HLA* alleles that did not reach significance. The light gray dotted line indicates nominal significance ($P$ =0.05). AF = allele frequency; OR = odds ratio; CI= confidence interval.

**Table 2.** HLA associations before and after adjusting for the primary association in juvenile idiopathic arthritis associated uveitis and non-juvenile idiopathic arthritis associated uveitis versus controls (n=499).

| JIA uveitis (n=92) | | | | | | | |
|---|---|---|---|---|---|---|---|
| **HLA allele** | **Univariable analysis** | | **Conditional analysis of HLA alleles** | | | | |
| | | | **Step 1: *DRB1*08:01* enters** | | **$r^2$ with DRB1* 08:01** | **Step 2: *DRB1*11:04* enters** | |
| | OR (95% CI) | *P* | OR (95% CI) | *P* | | OR (95% CI) | *P* |
| *HLA-DRB1*08:01* | 11.12 (5.76-21.43) | $6.51\times10^{-13}$ | Conditioned | | | Conditioned | |
| *HLA-DQB1*04:02* | 10.11 (5.32-19.22) | $1.68\times10^{-12}$ | 1.51 (0.09-25.16) | 0.78 | 0.86 | 1.46 (0.08-25.78) | 0.79 |
| *HLA-DRB1*11:04* | 5.63 (2.80-11.36) | $1.37\times10^{-6}$ | 4.65 (2.23-9.72) | $4.39\times10^{-5}$ | 0.01 | Conditioned | |

| Non-JIA uveitis (n=185) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **HLA allele** | **Univariable analysis** | | **Conditional analysis of HLA alleles** | | | | | | | | | |
| | | | **Step 1: *DRB1*01:02* enters** | | **$r^2$ with *DRB1*01:02*** | **Step 2: *DRB1*04:03* enters** | | **Step 3: *DQB1*04:02* enters** | | **Step 4: *DQB1*05:03* enters** | |
| | OR (95% CI) | *P* | OR (95% CI) | *P* | | OR (95% CI) | *P* | OR **(95% CI)** | *P* | OR **(95% CI)** | *P* |
| *HLA-DRB1*01:02* | 5.58 (2.70-11.55) | $3.60\times10^{-6}$ | Conditioned | | | Conditioned | | Conditioned | | Conditioned | |
| *HLA-DRB1*04:03* | 11.98 (4.01-35.76) | $8.48\times10^{-6}$ | 11.67 (3.82-35.66) | $1.62\times10^{-5}$ | 0.009 | Conditioned | | Conditioned | | Conditioned | |
| *HLA-DQB1*05:03* | 2.94 (1.69-5.09) | $1.23\times10^{-4}$ | 3.01 (1.72-5.25) | $1.06\times10^{-4}$ | 0.00 | 3.19 (1.82-5.60) | $5.09\times10^{-5}$ | 3.31 (1.87-5.83) | $3.65\times10^{-4}$ | Conditioned | |
| *HLA-DQB1*04:02* | 3.20 (1.71-6.02) | $2.96\times10^{-4}$ | 3.45 (1.83-6.52) | $1.37\times10^{-4}$ | 0.001 | 3.82 (2.01-7.25) | $4.21\times10^{-5}$ | Conditioned | | Conditioned | |
| *HLA-DRB1*14:01* | 0.58 (0.21-1.57) | 0.28 | 0.65 (0.24-1.77) | 0.40 | 0.001 | 0.71 (0.26-1.94) | 0.51 | 0.80 (0.29-2.19) | 0.66 | 0.01 (0.002-0.12) | $8.96\times10^{-5}$ |

Next, adjusting for *DRB1*01:02, DRB1*04:03,* and *DQB1*04:02* revealed an independent association for *HLA-DQB1*05:03* (OR = 3.31, 95% CI 1.87-5.83; $P = 3.65 \times 10^{-4}$). And finally, adjusting for *DRB1*01:02, DRB1*04:03*, *DQB1*04:02,* and *DQB1*05:03,* identified an independent association for *HLA-DRB1*14:01* (OR = 0.01, 95% CI 0.002-0.12; $P = 8.96 \times 10^{-5}$) (**Table 2**). After adjusting for *DRB1*01:02, DRB1*04:03*, *HLA-DQB1*04:02, DQB1*05:03*, and *DRB1*14:01,* no additional HLA-wide associations were detected ($P > 2.79 \times 10^{-4}$).

**The JIA risk *HLA-DRB1*08:01-HLA-DQB1*04:02* haplotype is associated with idiopathic chronic anterior uveitis**

Next, we stratified patients according to the anatomical location of uveitis. The frequency of the classical *HLA* alleles in all cases according to the anatomical location of their inflammation and controls are presented in **Supplementary table 4.**

Idiopathic chronic anterior uveitis (CAU) is phenotypically similar to JIA-U, albeit without clinical evidence of arthritis. Remarkably, when testing for 50 CAU cases versus controls, we observed an HLA-wide significant association for the JIA risk loci *HLA-DQB1*04:02* (OR = 5.37, 95% CI 2.38-12.09; $P = 5.07 \times 10^{-5}$) and *HLA-DRB1*08:01* (OR = 5.30, 95% CI 2.26-12.44; $P = 1.30 \times 10^{-4}$) (**Table 3, Figure 3A and Supplementary table 4**)**.** As expected, after adjusting for *HLA-DQB1*04:02,* the association signal for *HLA-DRB1*08:01* in CAU was lost, but in contrast to JIA-U revealed independent association for *HLA-DQB1*05:03* (OR = 5.86, 95% CI 2.52-13.62; $P = 3.92 \times 10^{-5}$) (**Figure 3B**). Further adjusting for *HLA-DQB1*05:03* removed the remainder of the signal (**Figure 3C**). Note that although we considered the number of cases with acute anterior uveitis too low (n=18) to be meaningful for disease-specific testing, the allele frequency of *HLA-DQB1*04:02* or *HLA-DRB1*08:01* was nearly identical to CAU, thus also higher compared to controls (**Supplementary table 4**)**.** In summary, these data show that the *HLA-DRB1*08:01-HLA-DQB1*04:02* haplotype is associated with pediatric anterior uveitis.

***HLA-DRB1*01:02*, *HLA-DRB1*04:03* and *HLA-DQB1*05:03* are risk alleles for pediatric panuveitis**

We did not observe an HLA-wide significant association for intermediate uveitis most likely due to the limited number of cases with this subtype in this study (n=39) but detected *HLA-DRB1*15:01* (OR = 2.18, 95% CI 1.26-3.77; $P = 0.005$) as the lead allele, which is in line with previous observations[11,12] (**Table 3 and supplementary Table 4**). We detected *HLA-DRB1*01:02* (OR = 10.18, 95% CI 4.55-22.76; $P = 1.58 \times 10^{-8}$) as the primary association in patients with panuveitis (**Table 3, Figure 3D,** and

**Supplementary table 4**). We then adjusted for *HLA-DRB1*01:02,* which revealed independent association for the rare allele *HLA-DRB1*04:03* (OR = 29.61, 95% CI 7.96-110.22; $P = 4.35 \times 10^{-7}$) (**Figure 3E**). Adjusting for both *DRB1*01:02* and *DRB1*04:03* we discovered an additional independent association for *HLA-DQB1*05:03* (OR = 4.51, 95% CI: 2.27-8.96; $P = 1.72 \times 10^{-5}$) (**Figure 3F**). Finally, conditioning on all three risk *HLA* alleles removed the remainder of the signal ($P > 2.79 \times 10^{-4}$). (**Figure 3G**). The haplotype *HLA-DRB1*01:02* and *HLA-DQB1*05* have been previously reported in patients suspected of TINU syndrome.[19-22] However, removing these patients (n=15) from the analysis modestly affected the signal (*HLA-DRB1*01:02*, $P = 1.61 \times 10^{-6}$, and *HLA-DQB1*05:03*, $P = 8.59 \times 10^{-4}$) and indicates that the association signal at DRB1*01:02 and DQB1*05:03 represents a signal beyond the association with TINU.

**Table 3.** Primary HLA associations in juvenile idiopathic arthritis associated uveitis, idiopathic chronic anterior uveitis (no JIA), intermediate uveitis and panuveitis versus controls (n=499).

| **JIA uveitis (n=92)** | | |
|---|---|---|
| **Univariable analysis** | | |
| **HLA allele** | OR (95% CI) | *P* |
| *HLA-DRB1*08:01* | 11.12 (5.76-21.43) | $6.51 \times 10^{-13}$ |
| *HLA-DQB1*04:02* | 10.11 (5.32-19.22) | $1.69 \times 10^{-12}$ |
| *HLA-DRB1*11:04* | 5.63 (2.80-11.36) | $1.37 \times 10^{-06}$ |
| **Idiopathic chronic anterior uveitis (no JIA, n=50)** | | |
| **Univariable analysis** | | |
| **HLA allele** | OR (95% CI) | *P* |
| *HLA-DQB1*04:02* | 5.37 (2.38-12.09) | $5.07 \times 10^{-05}$ |
| *HLA-DQB1*05:03* | 5.10 (2.25-11.58) | $9.73 \times 10^{-05}$ |
| *HLA-DRB1*08:01* | 5.30 (2.26-12.44) | $1.30 \times 10^{-04}$ |
| **Intermediate uveitis (n=39)** | | |
| **Univariable analysis** | | |
| **HLA allele** | OR (95% CI) | *P* |
| *HLA-DRB1*15:01* | 2.18 (1.26-3.77) | 0.005 |
| **Panuveitis (n=81)** | | |
| **Univariable analysis** | | |
| **HLA allele** | OR (95% CI) | *P* |
| *HLA-DRB1*01:02* | 10.18 (4.55-22.76) | $1.58 \times 10^{-08}$ |
| *HLA-DRB1*04:03* | 29.30 (8.39-102.41) | $1.22 \times 10^{-07}$ |
| *HLA-DQB1*05:03* | 3.71 (1.94-7.14) | $7.97 \times 10^{-05}$ |

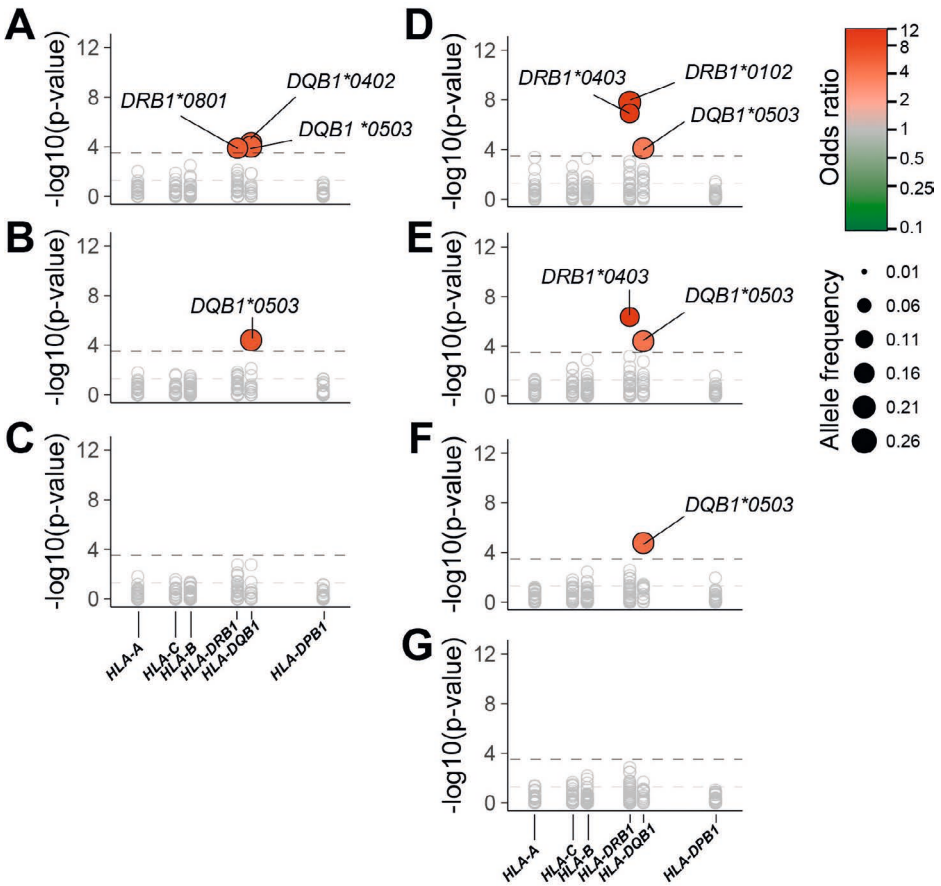


**Figure 3.** Independent *HLA* allele associations (i.e., conditional testing) in cases with idiopathic chronic anterior uveitis and panuveitis.

**A)** Initial association testing in 50 chronic anterior uveitis cases detected the primary association for *HLA-DQB1*04:02* (AF=0.10, OR =5.37, 95% CI 2.38-12.09; $P = 5.07 \times 10^{-5}$) **B)** Adjusting for lead *HLA* allele (*HLA-DQB1*04:02)*, revealed independent association for *HLA-DQB1*05:03* (AF=0.10, OR 5.86, 95% CI 2.52-13.62; $P = 3.92 \times 10^{-5}$). **C)** After adjusting for *HLA-DQB1*04:02-HLA-DQB1*05:03* no additional independent *HLA* associations were detected. **D)** Initial association testing in 81 panuveitis cases mapped the primary association to *HLA-DRB1*01:02* (AF=0.11, OR =10.18, 95% CI 4.55-22.76; $P = 1.58 \times 10^{-8}$). **E)** After adjusting for *HLA-DRB1*01:02* an independent association for *HLA-DRB1*04:03* (AF=0.07, OR =29.61, 95% CI 7.96-110.22; $P = 4.35 \times 10^{-7}$) was detected. **F)** Adjusting for *HLA-DRB1*01:02*, and *HLA-DRB1*04:03* discovered independent association for HLA-DQB1*05:03 (AF=0.10, OR = 4.51, 95% CI 2.27-8.96; $P = 1.72 \times 10^{-5}$). **G)** Adjusting for *HLA-DRB1*01:02, HLA-DRB1*04:03,* and *HLA-DQB1*05:03* revealed no additional independent *HLA* associations. The size and color of the dots represent the allele frequency and effect size (odds ratio), respectively. The gray dots represent the *HLA* alleles that did not reach significance. HLA-wide significance threshold ($P = 2.79 \times 10^{-4}$) is indicated as a dashed dark gray line in the association plots). AF = allele frequency; OR = odds ratio; CI= confidence interval.

**Sex-specific differences in associations with HLA alleles**

Because we previously detected sexual dimorphism in uveitis associated with JIA[14], we were interested to test whether males and females showed differential association with *HLA* alleles (**Supplementary table 5**). The distribution of male and females across each group is shown in **Table 4.** Several notable observations were made; considering 27 males with JIA-U versus 250 male controls, we detected association for a novel risk allele *HLA-DRB1*11:01* (OR = 6.25, 95% CI 2.49-15.72; $P = 9.71 \times 10^{-5}$) which was not associated with females ($P = 0.32$). In analysis of 65 females with JIA-U, we observed a significant association for *HLA-DRB1*11:04* (OR = 10.16, 95% CI 3.88-26.58; $P = 2.32 \times 10^{-6}$), which was not associated with males ($P = 0.21$).

Testing female patients (n=33) with CAU versus 249 female controls revealed *HLA-DQB1*05:03* (OR= 13, 95% CI 4.17-40.49; $P=9.75 \times 10^{-6}$) as the primary association over *HLA-DQB1*04:02* ($P = 4.95 \times 10^{-4}$). However, our cohort of CAU cases may not have sufficient power to accurately assign one risk allele over another. Therefore, we ran the association test at *HLA-DQB1*05:03* for CAU and included sex as an interaction term in the model. With this test you can examine if sex influences the *HLA* association in general. This analysis showed a moderate, but significant effect for the interaction term ($P_{interaction} = 0.03$), indicating that the *HLA-DQB1*05:03* signal was indeed sex specific. We also ran interaction tests for the two JIA-U sex-specific alleles *HLA-DRB1*11:01* and *HLA-DRB1*11:04*, which revealed similar interactions with sex ($P_{interaction} = 0.0006$ and $P_{interaction} = 0.06$, respectively). These data show sex specific differences in the association with risk *HLA* alleles.

**Table 4.** The distribution of males and females in the investigated groups.

| Diagnosis | Total number of cases | Number of males | Number of females |
|---|---|---|---|
| JIA associated uveitis, No. (%) | 92 (33) | 27 (29) | 65 (71) |
| Idiopathic chronic anterior uveitis, No. (%) | 50 (18) | 17 (34) | 33 (66) |
| Panuveitis, No. (%) | 81 (29) | 43 (53) | 38 (47) |

**HLA amino acid associations with uveitis**

Next, we analyzed the association of *HLA* amino acid polymorphisms with uveitis to assess the contribution of specific amino acid positions (**Supplementary table 1A-F and Supplementary table 6**). Univariate analysis identified 12 amino acids at 12 positions for JIA-U and CAU, eight amino acids at eight positions for IU, and

two amino acids at two positions for panuveitis (**Supplementary table 7A and 7B**). However, none of the amino acids showed a stronger association compared to the classical alleles. (**Supplementary table 7A**). In other words, no polymorphic amino acid position could explain the data better than the classical *HLA* alleles.

**Aspartic acid (D) at position 57 increases the peptide binding affinity to HLA-DQ**

We aimed to explore the functional implications of risk *HLA* alleles in pediatric uveitis. The amino acid position 86 distinguishes the JIA-U associated alleles *HLA-DRB1*11:01* (86-Glycine) in males, *HLA-DRB1*11:04* (86-Valine) in females (**Supplementary table 1E**). This position has been shown to control DR alpha/beta dimer stability, but also peptide binding for the panuveitis-associated *HLA-DRB1*04:03*, and is therefore likely to influence antigen presentation.[40,41] Therefore, we asked whether additional allele-defining positions affect the binding of peptides to HLA allotypes encoded by risk *HLA* alleles. The *HLA-DQB1*05:03* was found to be associated with two distinct forms of uveitis CAU and PAN, and this allele is functionally understudied compared to other uveitis risk alleles detected in this study. This allele harbors an aspartic acid (D) at position 57 while other non-risk alleles for *HLA-DQB1*05* have a serine (S) or valine (V) at position 57 (**Figure 4A**). Curiously, the amino acid position 57 of the DQ betachain is located at the edge of the peptide binding groove (**Figure 4A**) and confers risk to several autoimmune conditions suggesting it to be involved in peptide binding of HLA-DQ.[42-46] To assess whether the presence of aspartic acid at position 57 functionally affects binding of peptide cargo to HLA-DQ, we extracted ligands (n=157 from the HLA ligand atlas) from immunopeptidomes of *HLA-DQB1*05:01* (57-S), *HLA-DQB1*05:02* (57-V) and *HLA-DQB1*05:03* (57-D) that only differ in the amino acid residue at position 57 (**Supplementary table 8**).[35] Next, we modeled the binding affinity of the naturally occurring ligands to HLA-DQ, by using the full amino acid sequence of *HLA-DQB1*05* in NetMHCIIpan 4.0 and performed amino acid substitutions at position 57.[36] We observed that substitution of position 57 from S or V (nonrisk alleles) to the uveitis-associated amino acid aspartic acid (D) (equivalent to *DQB1*05:03*) resulted in a significant increased binding affinity score (S versus D; $P = 1.67 \times 10^{-27}$ and V versus D $P = 1.64 \times 10^{-27}$) (**Figure 4B**). In more detail, major binding pockets for HLA-DQ are position P4, P6 and P9 in the peptide motif.[47] Therefore, we computed the peptide motif of the 157 HLA-DQB1:05 ligands and the top 50 peptides with the largest difference in binding scores between HLA-*DQB1*05:02* (57-V) and *HLA-DQB1*05:03* (57-D) and compared the core sequence alignments to identify changes in specific amino acid position. This analysis revealed, as expected a marked change in amino acid residue

preference in particular at anchor position P9, which directly interacts with position 57 (**Figure 4C**). Collectively, these data show that position 57 in the HLA-DQ beta chain modulates the peptide binding capacity and indicates risk *HLA* alleles associated with pediatric uveitis show altered antigen presentation capacity.

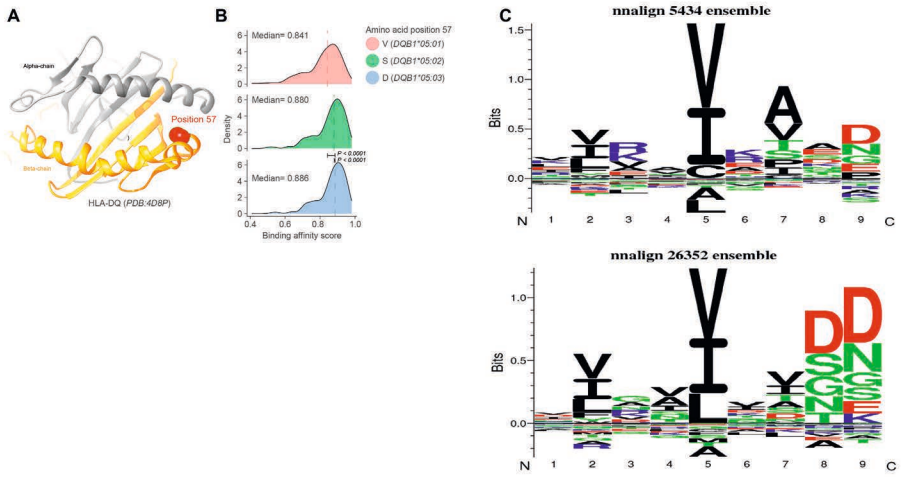


**Figure 4.** The effect of amino acid substitutions for position 57 on predicted binding affinity for natural ligands of HLA-DQ*05 alleles.

**A)** View into the peptide-binding groove of a three-dimensional ribbon model for HLA-DQB1 (based on Protein Data Bank entry 4D8P).[46] Position 57 that distinguishes *DQB1*05* alleles is highlighted in red. **B)** The effect of amino acid substitution for position 57 on predicted binding affinity for naturally presented ligands eluted from *HLA-DQB1*05:01, HLA-DQB1*05:02, and HLA-DQB1*05:03* (n=157, see **Supplementary table 8**). Binding affinity scores were predicted using the full amino acid sequence of *HLA-DQB1*05* in *NetMHCIIpan 4.0*.[36] **C)** A sequence logo representation of the peptide motif of the 157 *HLA-DQB1:05* peptides from the HLA atlas as computed by the NNAlign 2.0 server.[37] The relative height of color-coded amino acid (letter code) at each position represent the information content in bits. Positive values show favored amino acids at that position and negative bits indicate amino acids that are not preferred at that position. The anchor position P4, P6, and P9 of HLA-DQ are highlighted.

## DISCUSSION

The role of the human *MHC* locus in determination of the susceptibility to uveitis is evident from the routine screening for *HLA* alleles (more specifically *HLA-B27* and *HLA-A29*) to support the diagnosis of specific entities of noninfectious uveitis.[48] One of the outcomes of this study is that we detected a predominant contribution of

common alleles to disease risk that allowed accurate comparison with available data from population controls[28], but also detected the less common or *rare* allele *HLA-DRB1*04:03* (allele frequency of 0.7% in controls) as a novel risk allele for panuveitis. This supports that common and rare alleles define the susceptibility to uveitis in children.

Previous genome-wide association studies have consistently identified HLA class I and class II genes as the primary genetic risk factors for noninfectious uveitis.[10-15] However, these studies were based primarily on HLA imputation methods that despite their overall high accuracy to identify common alleles may be less adequate for the identification of rare or poorly characterized alleles. We therefore used sequencing data to comprehensively determine the full sequence at high resolution of class I and class II *HLA* alleles for a large cohort of patients with pediatric uveitis. Note imputed *HLA* alleles from population controls were nearly identical to the allele frequencies in Europe (**Supplementary table 9**).[49]

Our study revealed that *HLA-DQB1*04:02* and *HLA-DRB1*08:01* (in strong LD) are risk alleles for both JIA-U and CAU. Idiopathic chronic anterior uveitis is clinically similar to JIA-U uveitis, but these patients do not present with any evidence for inflammatory arthritis.[50] A possible explanation for the shared genetic alleles could be that in the minority of JIA-U cases, uveitis may first manifest before the onset of arthritis (classified as CAU). A fraction of CAU cases may later develop arthritis and will be eventually classified as JIA-U.[50,51] However, in some of them, arthritis development might be suppressed by immunomodulating treatment for uveitis. In contrast to a previous GWAS in JIA-U, here we did not include JIA patients without uveitis and therefore could not formally test whether the identified associations were related to uveitis or JIA. However, several lines of evidence link *DQB1*04:02-DRB1*08:01* to pediatric anterior uveitis. First, we showed that these alleles are genetically linked to CAU. Second, the allele frequency of these alleles in a previous GWAS was higher in patients with JIA-U compared to JIA patients without uveitis (0.19 vs 0.11, respectively).[14] Finally, we showed that the allele frequency of *HLA-DQB1*04:02* and *HLA-DRB1*08:01* was also increased in patients with acute anterior uveitis, in line with previous observations.[52] These observations suggests that despite the association of these alleles with JIA in general these alleles may contribute to a common molecular mechanism that promotes anterior uveitis, perhaps also in JIA-uveitis. [38,39] Future studies that use additional genotype data to fine-map the *MHC* will provide sufficient

resolution (extended haplotypes including noncoding polymorphisms) to perform bivariate analysis to determine the genetic correlation between these different types of anterior uveitis.

In addition, such studies should also aim to expand the cohort to represent non-European populations. A limitation of the current study is that cases and controls of the study were near exclusively from Western European ancestry as visualized by UMAP. Note that because UMAP dedicates relatively more visual space on populations with larger sample size, this may exaggerate the *variation* within cases and controls in the projection. Regardless, it remains to be determined if the here identified risk loci for anterior uveitis are shared with other patient populations. For example, in East Asian populations the prevalence of uveitis in the JIA populations may be different compared to Europe.[53]

We identified three new independent HLA-wide associations *HLA-DRB1*01:02, HLA-DRB1*04:03,* and *HLA-DQB1*05:03* as being strongly associated with panuveitis. *HLA-DRB1*01:02* and *HLA-DQB1*05* associations have been previously reported in patients suspected for TINU syndrome by Levinson and co-workers.[20] A follow-up study from the same group, however, showed that the *HLA-DRB1*0102* and *HLA-DRB1*08:01* alleles were associated with patients with isolated acute anterior uveitis, but not in those with isolated biopsy-proven TIN.[21] Perasaari *et al*. identified *HLA-DRB1*08:01* and *HLA-DRB1*14:01* as risk alleles rather than *HLA-DRB1*01:02* in patients with biopsy-proven TINU.[19] Our data also showed that in patients with acute anterior uveitis the allele frequency of *HLA-DRB1*08:01* was higher compared to controls (**Supplementary table 4)**. Of interest, work by Reddy *et al*. demonstrated that 14 out of 15 children with unexplained panuveitis also carried the *HLA-DRB1*01-HLA-DQB1*05* haplotype.[22] In our study, 15 of 81 patients with panuveitis were suspected for TINU syndrome. However, removing these patients (n=15) from the analysis modestly affected the signal and indicates that the association signal at DRB1*01:02 and DQB1*05:03 might represent a signal beyond the association with TINU. In other words, we speculate that the alleles *HLA-DRB1*01* and *HLA-DQB1*05* might convey risk for panuveitis and *HLA-DRB1*08:01* may convey risk for anterior uveitis. Though, it should be noted that TINU is often asynchronous and therefore TINU might be underestimated. We further note that the diagnosis of some subtypes of uveitis may be prone to unintentional heterogeneity due to the intrinsic dependency to classify pediatric uveitis based on clinical expert opinion. Regardless, the diagnosis of uveitis according

to the SUN criteria (as done in this study) may allow comparison between studies. These classification criteria appeared to perform well enough for use in clinical and translational research.[54] We detected *HLA-DRB1*15:01* as the lead allele in cases with IU, which is in line with previous observations.[11,12] Remarkably, this allele is also the primary association in multiple sclerosis, an inflammatory demyelinating disease of the central nervous system and is often accompanied by IU.[55] Furthermore, IU patients also show demyelinating lesions by MRI, supporting the close relation of IU and MS.[56] In our study, two cases that carried the *HLA-DRB1*15:01* allele showed demyelinating lesions by MRI.

We previously showed that uveitis in JIA is genetically linked to other amino acid positions by comparing to JIA without uveitis rather than an individual *HLA* allele.[14] In the current study, we demonstrated that the disease risk is detected best by the classical *HLA* alleles instead of specific amino acid positions, which are often shared by multiple *HLA* alleles. Alternatively, moderate-to-strong LD between *HLA* alleles and nearby non-HLA loci may suggest that risk *HLA* alleles serve as a *proxy* for variants in non-HLA genes implicated in pediatric uveitis. For example, HLA-DQB1:04 is in LD with the TAP1 gene involved in antigen presentation. [57]

Further analyses revealed that the association signals in JIA-U (*HLA-DQB1*04:02*), CAU (*HLA-DQB1*05:03*) and panuveitis (*HLA-DRB1*01:02)* showed evidence for sexual dimorphism. We identified male-specific associations for *HLA-DRB1*11:01* in JIA-U, which showed a large difference in frequency with females. Conversely, *HLA-DRB1*11:04* was only associated with females. Our data supported sex-specific effects based on interaction of sex with the risk for these alleles. Sex-specific *HLA* allele associations have been reported in other chronic inflammatory conditions.[14,58-60] This is in line with functional data on sex differences in the adaptive immune responses and the degree to which HLA molecules interact with T cells (i.e. selection and expansion). [61-63]

Other functional implications of our data became evident by functional modeling of allele-defining amino acid positions. Although such studies have been done more extensively for HLA-DR.[40,41] Since we previously showed that amino acid positions 10 to 12 encoded by the *HLA–DRB1* gene are the primary genetic risk factors for uveitis in JIA[14], we focused in our study on *HLA DQB1*05:03* especially since it revealed association with multiple clinically distinct types of uveitis. We demonstrated similar

direct effects on peptide presentation for HLA-DQ. We showed that the risk allele *HLA-DQB1*05:03* was found to be associated with two forms of uveitis CAU and PAN. This allele harbors an aspartic acid (D) at position 57 while the other HLA-DQB1*05 alleles have a serine (S) or valine (V) at that position. We observed that amino acid substitution of position 57 from S or V (in the nonuveitis associated allele *HLA-DQB1*05:01* and *HLA-DQB1*05:02*) to the uveitis-associated amino acid aspartic acid (D) in *DQB1*05:03* increased the binding of peptides, underscoring the critical involvement of this position in antigen presentation by HLA-DQ. Alternatively, although not evaluated in this study, this position may also influence the flexibility and possibility to accommodate longer peptides protruding out of the groove.

In conclusion, we conducted a comprehensive analysis of *HLA* alleles implicated in uveitis susceptibility in children. We ascertained previous and identified novel independent *HLA* associations. We further demonstrated that the phenotypically similar CAU and JIA-U share *HLA* associated alleles. This suggests a common molecular basis for these types of uveitis and might help to understand the molecular mechanism driving pediatric uveitis.

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## SUPPLEMENTARY DATA

### Supplementary tables

**Supplementary table 1A**: Polymorphic amino acid positions in the *HLA-A* gene used in this study.

WEBLINK: https://iovs.arvojournals.org/article.aspx?articleid=2776464

**Supplementary table 1B**: Polymorphic amino acid positions in the *HLA-B* gene used in this study.

WEBLINK: https://iovs.arvojournals.org/article.aspx?articleid=2776464

**Supplementary table 1C**: Polymorphic amino acid positions in the *HLA-C* gene used in this study.

WEBLINK: https://iovs.arvojournals.org/article.aspx?articleid=2776464

**Supplementary table 1D**: Polymorphic amino acid positions in the *HLA-DPB1* gene used in this study.

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**Supplementary table 1E**: Polymorphic amino acid positions in the *HLA-DRB1* gene used in this study.

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**Supplementary table 1F**: Polymorphic amino acid positions in the *HLA-DQB1* gene used in this study.

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**Supplementary table 2:** Ancestry populations from the 1000 Genomes Project Consortium.[32,33]

| Population code | Population description | Super population code |
|---|---|---|
| CHB | Han Chinese in Beijing, China | East Asian |
| JPT | Japanese in Tokyo, Japan | East Asian |
| CHS | Southern Han Chinese | East Asian |
| CHD | Chinese in Metropolian Denver, USA | East Asian |
| CEU | Utah Residents (CEPH) with Northern and Western European Ancestry | European |
| TSI | Toscani in Italia | European |
| FIN | Finnish in Finland | European |
| GBR | British in England and Scotland | European |
| YRI | Yoruba in Ibadan, Nigeria | African |
| LWK | Luhya in Webuye, Kenya | African |
| ASW | Americans of African Ancestry in southwestern USA | African |
| MXL | Mexican Ancestry from Los Angeles USA | Mixed American |
| PUR | Puerto Ricans from Puerto Rico | Mixed American |
| CLM | Colombians from Medellin, Colombia | Mixed American |

**Supplementary table 3:** The allele frequency and summary stats of association testing of all uveitis cases (n=280) versus healthy controls (n=499).

| Classical Allele | Frequency | | OR | 95% CI | P-value |
|---|---|---|---|---|---|
| | Cases | Controls | | | |
| *HLA-A *0101* | 0.15 | 0.2 | 0.68 | (0.51-0.9) | 0.008 |
| *HLA-A *0103* | 0.00 | 0.00 | 0.51 | (0.03-8.24) | 0.637 |
| *HLA-A *0201* | 0.3 | 0.29 | 1.04 | (0.83-1.29) | 0.761 |
| *HLA-A *0202* | 0.00 | 0.00 | 3.38 | (0.08-152.21) | 0.53 |
| *HLA-A *0205* | 0.01 | 0.01 | 2.43 | (0.75-7.87) | 0.139 |
| *HLA-A *0206* | 0.01 | 0.00 | 1.43 | (0.29-7.17) | 0.663 |
| *HLA-A *0207* | 0.00 | 0.00 | 2.69 | (0.06-127.73) | 0.615 |
| *HLA-A *0211* | 0.00 | 0.00 | 2.65 | (0.06-123.61) | 0.62 |
| *HLA-A *0217* | 0.00 | 0.00 | 1.4 | (0.05-42.73) | 0.847 |
| *HLA-A *0301* | 0.12 | 0.16 | 0.71 | (0.52-0.97) | 0.033 |
| *HLA-A *0302* | 0.01 | 0.00 | 16.83 | (0.81-350.44) | 0.068 |
| *HLA-A *1101* | 0.06 | 0.05 | 1.2 | (0.75-1.93) | 0.447 |
| *HLA-A *2301* | 0.01 | 0.02 | 0.58 | (0.25-1.39) | 0.226 |
| *HLA-A *2402* | 0.09 | 0.08 | 1.21 | (0.84-1.74) | 0.311 |
| *HLA-A *2407* | 0.00 | 0.00 | 1.48 | (0.04-57.49) | 0.835 |
| *HLA-A *2501* | 0.01 | 0.00 | 2.98 | (0.33-26.62) | 0.329 |
| *HLA-A *2601* | 0.03 | 0.03 | 1.03 | (0.56-1.91) | 0.921 |
| *HLA-A *2902* | 0.01 | 0.02 | 0.83 | (0.34-2.01) | 0.675 |
| *HLA-A *3001* | 0.02 | 0.01 | 1.57 | (0.68-3.62) | 0.29 |
| *HLA-A *3002* | 0.01 | 0.00 | 1.47 | (0.36-5.92) | 0.591 |
| *HLA-A *3004* | 0.01 | 0.00 | 3.55 | (0.46-27.62) | 0.226 |
| *HLA-A *3101* | 0.02 | 0.04 | 0.54 | (0.27-1.07) | 0.076 |
| *HLA-A *3201* | 0.03 | 0.04 | 0.72 | (0.39-1.32) | 0.286 |
| *HLA-A *3301* | 0.01 | 0.00 | 3.28 | (0.91-11.73) | 0.068 |
| *HLA-A *3303* | 0.01 | 0.00 | 3.41 | (0.7-16.66) | 0.129 |
| *HLA-A *3402* | 0.00 | 0.00 | 7.99 | (0.26-245.47) | 0.234 |
| *HLA-A *3601* | 0.00 | 0.00 | 2.88 | (0.06-143.33) | 0.596 |
| *HLA-A *6601* | 0.00 | 0.00 | 7.96 | (0.26-243.13) | 0.234 |
| *HLA-A *6801* | 0.05 | 0.03 | 1.52 | (0.92-2.52) | 0.105 |
| *HLA-A *6802* | 0.00 | 0.00 | 2.38 | (0.25-22.57) | 0.45 |
| *HLA-A *6803* | 0.00 | 0.00 | 2.24 | (0.05-99.67) | 0.677 |
| *HLA-A *7401* | 0.01 | 0.00 | 10.16 | (0.46-225.44) | 0.143 |
| *HLA-B *0702* | 0.07 | 0.14 | 0.46 | (0.32-0.68) | 6.83E-05 |
| *HLA-B *0705* | 0.01 | 0 | 4.2 | (0.54-32.71) | 0.171 |
| *HLA-B *0801* | 0.11 | 0.14 | 0.74 | (0.53-1.02) | 0.069 |
| *HLA-B *1302* | 0.01 | 0.02 | 0.46 | (0.18-1.14) | 0.093 |
| *HLA-B *1401* | 0.00 | 0.00 | 0.63 | (0.06-6.24) | 0.695 |
| *HLA-B *1402* | 0.02 | 0.02 | 1.22 | (0.59-2.54) | 0.59 |
| *HLA-B *1501* | 0.07 | 0.09 | 0.7 | (0.47-1.04) | 0.078 |
| *HLA-B *1503* | 0.01 | 0.00 | 229.85 | (0-102305000) | 0.413 |
| *HLA-B *1510* | 0.00 | 0.00 | 68.07 | (0-39424000) | 0.533 |
| *HLA-B *1516* | 0.00 | 0.00 | 133.52 | (0-56692500) | 0.459 |
| *HLA-B *1517* | 0.01 | 0.01 | 1.52 | (0.4-5.74) | 0.538 |
| *HLA-B *1518* | 0.00 | 0.00 | 0.78 | (0.06-9.55) | 0.847 |

Supplementary table 3. (continued)

| Classical Allele | Frequency | | OR | 95% CI | P-value |
|---|---|---|---|---|---|
| | Cases | Controls | | | |
| *HLA-B *1801* | 0.06 | 0.03 | 1.77 | (1.08-2.89) | 0.023 |
| *HLA-B *2702* | 0.00 | 0.00 | 1.42 | (0.19-10.4) | 0.733 |
| *HLA-B *2704* | 0.00 | 0.00 | 56.99 | (0-1899570) | 0.447 |
| *HLA-B *2705* | 0.04 | 0.03 | 1.7 | (0.94-3.07) | 0.078 |
| *HLA-B *3501* | 0.08 | 0.07 | 1.22 | (0.82-1.82) | 0.33 |
| *HLA-B *3502* | 0.01 | 0.00 | 1.82 | (0.53-6.31) | 0.345 |
| *HLA-B *3503* | 0.02 | 0.02 | 1.22 | (0.54-2.78) | 0.63 |
| *HLA-B *3508* | 0.01 | 0.00 | 3.2 | (0.64-15.99) | 0.156 |
| *HLA-B *3543* | 0.00 | 0.00 | 7.02 | (0.02-2105.84) | 0.503 |
| *HLA-B *3701* | 0.02 | 0.02 | 0.77 | (0.35-1.73) | 0.532 |
| *HLA-B *3801* | 0.03 | 0.01 | 2.42 | (1.14-5.15) | 0.022 |
| *HLA-B *3802* | 0.00 | 0.00 | 0.8 | (0.07-9.74) | 0.863 |
| *HLA-B *3901* | 0.02 | 0.01 | 1.75 | (0.76-4.05) | 0.19 |
| *HLA-B *3906* | 0.01 | 0.01 | 1.43 | (0.51-4.01) | 0.491 |
| *HLA-B *4001* | 0.06 | 0.06 | 0.93 | (0.61-1.42) | 0.733 |
| *HLA-B *4002* | 0.01 | 0.02 | 0.66 | (0.27-1.63) | 0.373 |
| *HLA-B *4006* | 0.01 | 0.00 | 215.06 | (0.01-8239260) | 0.319 |
| *HLA-B *4101* | 0.01 | 0.00 | 64.24 | (0.36-11495.6) | 0.116 |
| *HLA-B *4102* | 0.00 | 0.00 | 1.09 | (0.07-16.43) | 0.952 |
| *HLA-B *4201* | 0.00 | 0.00 | 116.4 | (0-3286530) | 0.363 |
| *HLA-B *4202* | 0.00 | 0.00 | 57.27 | (0-1877150) | 0.445 |
| *HLA-B *4402* | 0.09 | 0.08 | 1.08 | (0.74-1.58) | 0.688 |
| *HLA-B *4403* | 0.02 | 0.05 | 0.5 | (0.27-0.91) | 0.023 |
| *HLA-B *4405* | 0.00 | 0.00 | 2.06 | (0.07-60.32) | 0.675 |
| *HLA-B *4501* | 0.00 | 0.00 | 1.22 | (0.16-9.6) | 0.85 |
| *HLA-B *4601* | 0.00 | 0.00 | 29.63 | (0-235884) | 0.46 |
| *HLA-B *4701* | 0.01 | 0.00 | 3.43 | (0.63-18.58) | 0.153 |
| *HLA-B *4901* | 0.01 | 0.01 | 1.52 | (0.5-4.59) | 0.457 |
| *HLA-B *5001* | 0.01 | 0.01 | 0.95 | (0.3-3) | 0.929 |
| *HLA-B *5101* | 0.06 | 0.06 | 1.06 | (0.67-1.67) | 0.802 |
| *HLA-B *5105* | 0.01 | 0.00 | 12.47 | (0.47-328.62) | 0.131 |
| *HLA-B *5108* | 0.00 | 0.00 | 7.12 | (0.08-632.91) | 0.391 |
| *HLA-B *5301* | 0.01 | 0.00 | 5.48 | (0.59-50.53) | 0.133 |
| *HLA-B *5501* | 0.01 | 0.02 | 0.76 | (0.32-1.77) | 0.52 |
| *HLA-B *5601* | 0.01 | 0.01 | 1.23 | (0.37-4.03) | 0.734 |
| *HLA-B *5701* | 0.01 | 0.03 | 0.4 | (0.17-0.92) | 0.032 |
| *HLA-B *5702* | 0.00 | 0.00 | 12.42 | (0.03-5518.31) | 0.418 |
| *HLA-B *5703* | 0.00 | 0.00 | 1.73 | (0.09-32.29) | 0.713 |
| *HLA-B *5801* | 0.01 | 0.01 | 1.19 | (0.37-3.83) | 0.772 |
| *HLA-C *0102* | 0.03 | 0.03 | 1.28 | (0.7-2.35) | 0.418 |
| *HLA-C *0202* | 0.05 | 0.05 | 1.05 | (0.64-1.73) | 0.834 |
| *HLA-C *0302* | 0.00 | 0.01 | 0.46 | (0.09-2.45) | 0.362 |
| *HLA-C *0303* | 0.08 | 0.07 | 1.08 | (0.72-1.6) | 0.715 |
| *HLA-C *0304* | 0.07 | 0.11 | 0.57 | (0.39-0.85) | 0.005 |

Supplementary table 3. (continued)

| Classical Allele | Frequency | | OR | 95% CI | P-value |
|---|---|---|---|---|---|
| | Cases | Controls | | | |
| *HLA-C *0305* | 0.00 | 0.00 | 1.08 | (0.06-18.46) | 0.958 |
| *HLA-C *0401* | 0.13 | 0.11 | 1.26 | (0.92-1.72) | 0.148 |
| *HLA-C *0501* | 0.07 | 0.07 | 1.07 | (0.7-1.62) | 0.766 |
| *HLA-C *0602* | 0.06 | 0.09 | 0.64 | (0.42-0.98) | 0.042 |
| *HLA-C *0701* | 0.15 | 0.17 | 0.89 | (0.67-1.18) | 0.417 |
| *HLA-C *0702* | 0.11 | 0.16 | 0.67 | (0.48-0.92) | 0.013 |
| *HLA-C *0704* | 0.03 | 0.02 | 1.29 | (0.64-2.6) | 0.47 |
| *HLA-C *0802* | 0.02 | 0.02 | 0.88 | (0.43-1.78) | 0.712 |
| *HLA-C *1202* | 0.00 | 0.00 | 0.74 | (0.13-4.35) | 0.743 |
| *HLA-C *1203* | 0.06 | 0.03 | 1.76 | (1.08-2.88) | 0.023 |
| *HLA-C *1402* | 0.02 | 0.01 | 2.18 | (0.98-4.86) | 0.057 |
| *HLA-C *1502* | 0.03 | 0.02 | 1.24 | (0.64-2.38) | 0.522 |
| *HLA-C *1505* | 0.01 | 0.00 | 3.95 | (0.94-16.62) | 0.061 |
| *HLA-C *1601* | 0.01 | 0.04 | 0.24 | (0.1-0.6) | 0.002 |
| *HLA-C *1602* | 0.01 | 0.01 | 1.11 | (0.29-4.2) | 0.874 |
| *HLA-C *1604* | 0.01 | 0.00 | 2.14 | (0.46-10.06) | 0.334 |
| *HLA-C *1701* | 0.01 | 0.01 | 3.27 | (1.01-10.6) | 0.048 |
| *HLA-DPB1 *0101* | 0.06 | 0.09 | 0.73 | (0.48-1.09) | 0.125 |
| *HLA-DPB1 *0201* | 0.18 | 0.15 | 1.24 | (0.93-1.66) | 0.134 |
| *HLA-DPB1 *0202* | 0.01 | 0.01 | 0.63 | (0.15-2.66) | 0.533 |
| *HLA-DPB1 *0301* | 0.08 | 0.11 | 0.76 | (0.52-1.11) | 0.154 |
| *HLA-DPB1 *0401* | 0.36 | 0.41 | 0.82 | (0.66-1.02) | 0.079 |
| *HLA-DPB1 *0402* | 0.1 | 0.11 | 0.9 | (0.64-1.27) | 0.55 |
| *HLA-DPB1 *0501* | 0.01 | 0.01 | 0.87 | (0.32-2.39) | 0.787 |
| *HLA-DPB1 *0601* | 0.01 | 0.02 | 0.33 | (0.09-1.22) | 0.097 |
| *HLA-DPB1 *0901* | 0.02 | 0.01 | 1.9 | (0.72-5.02) | 0.199 |
| *HLA-DPB1 *1001* | 0.01 | 0.02 | 0.59 | (0.21-1.68) | 0.322 |
| *HLA-DPB1 *1101* | 0.01 | 0.02 | 0.87 | (0.36-2.09) | 0.757 |
| *HLA-DPB1 *1301* | 0.01 | 0.01 | 0.63 | (0.22-1.8) | 0.387 |
| *HLA-DPB1 *1401* | 0.02 | 0.01 | 2.16 | (0.96-4.82) | 0.062 |
| *HLA-DPB1 *1501* | 0.01 | 0.01 | 0.89 | (0.2-3.86) | 0.872 |
| *HLA-DPB1 *1601* | 0.01 | 0.01 | 1.72 | (0.63-4.67) | 0.29 |
| *HLA-DPB1 *1701* | 0.01 | 0.01 | 0.94 | (0.39-2.31) | 0.898 |
| *HLA-DPB1 *1901* | 0.01 | 0.01 | 0.59 | (0.18-1.88) | 0.37 |
| *HLA-DPB1 *2001* | 0.00 | 0.01 | 0 | (0-7.67) | 0.148 |
| *HLA-DPB1 *2301* | 0.01 | 0.01 | 0.91 | (0.16-5.1) | 0.916 |
| *HLA-DPB1 *2601* | 0.00 | 0.00 | 2.61 | (0.25-27.55) | 0.426 |
| *HLA-DPB1 *3401* | 0.00 | 0.00 | 1.26 | (0.07-21.46) | 0.873 |
| *HLA-DPB1 *4501* | 0.00 | 0.00 | 1.07 | (0.07-16.79) | 0.959 |
| *HLA-DPB1 *8501* | 0.00 | 0.00 | 0.96 | (0.06-15.04) | 0.977 |
| *HLA-DQB1 *0201* | 0.13 | 0.15 | 0.87 | (0.64-1.18) | 0.376 |
| *HLA-DQB1 *0202* | 0.03 | 0.08 | 0.35 | (0.2-0.61) | 0.0002 |
| *HLA-DQB1 *0301* | 0.18 | 0.15 | 1.23 | (0.94-1.6) | 0.139 |
| *HLA-DQB1 *0302* | 0.09 | 0.11 | 0.87 | (0.61-1.24) | 0.439 |

Supplementary table 3. (continued)

| Classical Allele | Frequency | | OR | 95% CI | P-value |
|---|---|---|---|---|---|
| | Cases | Controls | | | |
| *HLA-DQB1 *0303* | 0.03 | 0.05 | 0.71 | (0.41-1.24) | 0.228 |
| *HLA-DQB1 *0305* | 0.01 | 0.00 | 74.6 | (0.09-63721.8) | 0.211 |
| *HLA-DQB1 *0402* | 0.09 | 0.02 | 5.27 | (3.04-9.12) | 2.98E-09 |
| *HLA-DQB1 *0501* | 0.11 | 0.11 | 0.99 | (0.71-1.38) | 0.951 |
| *HLA-DQB1 *0502* | 0.03 | 0.02 | 1.45 | (0.72-2.93) | 0.301 |
| *HLA-DQB1 *0503* | 0.06 | 0.03 | 2.25 | (1.33-3.82) | 0.003 |
| *HLA-DQB1 *0601* | 0.00 | 0.00 | 0.91 | (0.16-5.08) | 0.912 |
| *HLA-DQB1 *0602* | 0.08 | 0.13 | 0.58 | (0.41-0.83) | 0.003 |
| *HLA-DQB1 *0603* | 0.1 | 0.07 | 1.42 | (0.98-2.08) | 0.067 |
| *HLA-DQB1 *0604* | 0.04 | 0.08 | 0.51 | (0.31-0.83) | 0.007 |
| *HLA-DQB1 *0609* | 0.00 | 0.01 | 0.2 | (0.02-1.76) | 0.147 |
| *HLA-DRB1 *0101* | 0.05 | 0.04 | 1.55 | (0.9-2.67) | 0.112 |
| *HLA-DRB1 *0102* | 0.04 | 0.01 | 3.72 | (1.83-7.56) | 0.0003 |
| *HLA-DRB1 *0103* | 0.01 | 0.05 | 0.1 | (0.03-0.31) | 4.91E-05 |
| *HLA-DRB1 *0301* | 0.13 | 0.14 | 0.91 | (0.67-1.25) | 0.566 |
| *HLA-DRB1 *0302* | 0.00 | 0.00 | 7.18 | (0.09-586.1) | 0.38 |
| *HLA-DRB1 *0401* | 0.04 | 0.09 | 0.44 | (0.27-0.72) | 0.001 |
| *HLA-DRB1 *0402* | 0.02 | 0.00 | 4.16 | (1.25-13.89) | 0.02 |
| *HLA-DRB1 *0403* | 0.03 | 0.01 | 6.46 | (2.24-18.63) | 0.001 |
| *HLA-DRB1 *0404* | 0.02 | 0.03 | 0.4 | (0.18-0.9) | 0.026 |
| *HLA-DRB1 *0405* | 0.01 | 0.00 | 2.7 | (0.51-14.27) | 0.241 |
| *HLA-DRB1 *0407* | 0.01 | 0.01 | 0.46 | (0.12-1.81) | 0.266 |
| *HLA-DRB1 *0408* | 0.00 | 0.01 | 0.12 | (0.01-2.38) | 0.163 |
| *HLA-DRB1 *0701* | 0.04 | 0.11 | 0.36 | (0.23-0.57) | 1.42E-05 |
| *HLA-DRB1 *0801* | 0.08 | 0.02 | 5.21 | (2.94-9.23) | 1.53E-08 |
| *HLA-DRB1 *0802* | 0.00 | 0.00 | 8.23 | (0.08-827.82) | 0.37 |
| *HLA-DRB1 *0803* | 0.01 | 0.00 | 3.42 | (0.87-13.52) | 0.079 |
| *HLA-DRB1 *0804* | 0.01 | 0.00 | 6.42 | (0.65-63.63) | 0.112 |
| *HLA-DRB1 *0806* | 0.00 | 0.00 | 3.01 | (0.06-159.81) | 0.586 |
| *HLA-DRB1 *0901* | 0.02 | 0.02 | 1.13 | (0.54-2.34) | 0.746 |
| *HLA-DRB1 *1001* | 0.01 | 0.00 | 1.58 | (0.39-6.38) | 0.521 |
| *HLA-DRB1 *1101* | 0.07 | 0.06 | 1.2 | (0.77-1.88) | 0.412 |
| *HLA-DRB1 *1102* | 0.01 | 0.00 | 2.57 | (0.41-16.06) | 0.314 |
| *HLA-DRB1 *1103* | 0.01 | 0.00 | 2.46 | (0.56-10.8) | 0.232 |
| *HLA-DRB1 *1104* | 0.05 | 0.02 | 2.56 | (1.43-4.56) | 0.001 |
| *HLA-DRB1 *1201* | 0.02 | 0.01 | 1.16 | (0.48-2.79) | 0.745 |
| *HLA-DRB1 *1301* | 0.09 | 0.09 | 1.01 | (0.7-1.47) | 0.946 |
| *HLA-DRB1 *1302* | 0.05 | 0.07 | 0.71 | (0.44-1.13) | 0.148 |
| *HLA-DRB1 *1303* | 0.01 | 0.01 | 1.2 | (0.46-3.1) | 0.714 |
| *HLA-DRB1 *1305* | 0.00 | 0.00 | 2.13 | (0.05-88.86) | 0.692 |
| *HLA-DRB1 *1401* | 0.01 | 0.03 | 0.37 | (0.14-1.01) | 0.052 |
| *HLA-DRB1 *1404* | 0.01 | 0.01 | 0.85 | (0.13-5.72) | 0.864 |
| *HLA-DRB1 *1407* | 0.00 | 0.00 | 3.41 | (0.06-197.98) | 0.554 |
| *HLA-DRB1 *1501* | 0.08 | 0.14 | 0.58 | (0.41-0.83) | 0.003 |

Supplementary table 3. (continued)

| Classical Allele | Frequency | | OR | 95% CI | P-value |
|---|---|---|---|---|---|
| | Cases | Controls | | | |
| *HLA-DRB1 *1502* | 0.00 | 0.00 | 0.85 | (0.15-4.84) | 0.857 |
| *HLA-DRB1 *1601* | 0.01 | 0.02 | 0.6 | (0.22-1.58) | 0.297 |
| *HLA-DRB1 *1602* | 0.01 | 0.00 | 9.95 | (0.8-122.92) | 0.073 |

**Supplementary table 4:** The allele frequency (allele frequency ≥0.01) and association in cases according to the anatomical location of their inflammation versus healthy controls (n=499).

| Classical Allele | Frequency | | OR | 95% CI | P-value |
|---|---|---|---|---|---|
| | Cases | Controls | | | |
| **JIA uveitis (n=92)** | | | | | |
| *HLA-A *0101* | 0.10 | 0.2 | 0.43 | (0.25-0.73) | 1.75E-03 |
| *HLA-A *0201* | 0.41 | 0.29 | 1.76 | (1.26-2.45) | 8.27E-03 |
| *HLA-A *0205* | 0.01 | 0.01 | 1.11 | (0.11-11.71) | 0.930 |
| *HLA-A *0301* | 0.09 | 0.16 | 0.47 | (0.27-0.82) | 0.008 |
| *HLA-A *1101* | 0.05 | 0.05 | 1.1 | (0.53-2.3) | 0.799 |
| *HLA-A *2301* | 0.02 | 0.02 | 0.74 | (0.21-2.58) | 0.639 |
| *HLA-A *2402* | 0.09 | 0.08 | 1.13 | (0.64-1.99) | 0.68 |
| *HLA-A *2601* | 0.02 | 0.03 | 0.71 | (0.23-2.19) | 0.554 |
| *HLA-A *2902* | 0.01 | 0.02 | 0.78 | (0.16-3.91) | 0.762 |
| *HLA-A *3001* | 0.03 | 0.01 | 2.29 | (0.76-6.85) | 0.140 |
| *HLA-A *3101* | 0.02 | 0.04 | 0.41 | (0.12-1.38) | 0.149 |
| *HLA-A *3201* | 0.05 | 0.04 | 1.12 | (0.49-2.56) | 0.791 |
| *HLA-A *6801* | 0.05 | 0.03 | 1.52 | (0.7-3.28) | 0.230 |
| *HLA-B *0702* | 0.08 | 0.14 | 0.51 | (0.28-0.91) | 0.024 |
| *HLA-B *0801* | 0.07 | 0.14 | 0.43 | (0.23-0.79) | 0.007 |
| *HLA-B *1302* | 0.02 | 0.02 | 0.98 | (0.33-2.94) | 0.971 |
| *HLA-B *1402* | 0.01 | 0.02 | 0.3 | (0.04-2.34) | 0.250 |
| *HLA-B *1501* | 0.07 | 0.09 | 0.66 | (0.35-1.24) | 0.201 |
| *HLA-B *1517* | 0.01 | 0.01 | 1.28 | (0.14-11.62) | 0.823 |
| *HLA-B *1801* | 0.08 | 0.03 | 2.47 | (1.32-4.63) | 0.005 |
| *HLA-B *2705* | 0.03 | 0.03 | 1.43 | (0.55-3.67) | 0.462 |
| *HLA-B *3501* | 0.07 | 0.07 | 1.12 | (0.59-2.11) | 0.734 |
| *HLA-B *3503* | 0.03 | 0.02 | 2.41 | (0.85-6.78) | 0.096 |
| *HLA-B *3701* | 0.01 | 0.02 | 0.52 | (0.11-2.31) | 0.386 |
| *HLA-B *3801* | 0.03 | 0.01 | 3.38 | (1.23-9.29) | 0.018 |
| *HLA-B *3901* | 0.03 | 0.01 | 2.56 | (0.89-7.31) | 0.080 |
| *HLA-B *3906* | 0.03 | 0.01 | 3.88 | (1.28-11.72) | 0.016 |
| *HLA-B *4001* | 0.1 | 0.06 | 1.53 | (0.88-2.65) | 0.132 |
| *HLA-B *4002* | 0.02 | 0.02 | 1.2 | (0.38-3.73) | 0.757 |
| *HLA-B *4402* | 0.09 | 0.08 | 1.12 | (0.64-1.97) | 0.698 |
| *HLA-B *4403* | 0.01 | 0.05 | 0.22 | (0.05-0.93) | 0.039 |
| *HLA-B *4901* | 0.01 | 0.01 | 0.77 | (0.09-6.59) | 0.814 |
| *HLA-B *5001* | 0.01 | 0.01 | 1.24 | (0.24-6.48) | 0.800 |
| *HLA-B *5101* | 0.04 | 0.06 | 0.69 | (0.32-1.52) | 0.363 |
| *HLA-B *5501* | 0.01 | 0.02 | 0.57 | (0.13-2.62) | 0.473 |
| *HLA-B *5601* | 0.02 | 0.01 | 2.08 | (0.5-8.63) | 0.315 |
| *HLA-B *5701* | 0.01 | 0.03 | 0.17 | (0.02-1.31) | 0.089 |
| *HLA-B *5801* | 0.01 | 0.01 | 1.38 | (0.27-7.01) | 0.700 |
| *HLA-C *0102* | 0.05 | 0.03 | 1.96 | (0.87-4.44) | 0.106 |
| *HLA-C *0202* | 0.04 | 0.05 | 0.84 | (0.36-1.93) | 0.675 |
| *HLA-C *0302* | 0.01 | 0.01 | 0.77 | (0.08-7.04) | 0.815 |
| *HLA-C *0303* | 0.08 | 0.07 | 1.06 | (0.57-1.99) | 0.847 |

Supplementary table 4. (continued)

| Classical Allele | Frequency | | OR | 95% CI | P-value |
|---|---|---|---|---|---|
| | Cases | Controls | | | |
| *HLA-C *0304* | 0.1 | 0.11 | 0.8 | (0.47-1.37) | 0.411 |
| *HLA-C *0401* | 0.15 | 0.11 | 1.49 | (0.95-2.32) | 0.081 |
| *HLA-C *0501* | 0.07 | 0.07 | 1.09 | (0.57-2.07) | 0.795 |
| *HLA-C *0602* | 0.05 | 0.09 | 0.63 | (0.31-1.25) | 0.183 |
| *HLA-C *0701* | 0.12 | 0.17 | 0.68 | (0.42-1.1) | 0.117 |
| *HLA-C *0702* | 0.14 | 0.16 | 0.84 | (0.53-1.34) | 0.474 |
| *HLA-C *0704* | 0.03 | 0.02 | 1.21 | (0.43-3.41) | 0.717 |
| *HLA-C *0802* | 0.01 | 0.02 | 0.42 | (0.09-1.96) | 0.268 |
| *HLA-C *1203* | 0.08 | 0.03 | 2.46 | (1.31-4.63) | 0.005 |
| *HLA-C *1402* | 0.02 | 0.01 | 1.54 | (0.4-5.87) | 0.526 |
| *HLA-C *1502* | 0.02 | 0.02 | 0.6 | (0.17-2.14) | 0.427 |
| *HLA-C *1601* | 0.01 | 0.04 | 0.26 | (0.06-1.16) | 0.077 |
| *HLA-C *1602* | 0.01 | 0.01 | 0.71 | (0.07-6.82) | 0.765 |
| *HLA-C *1701* | 0.01 | 0.01 | 1.23 | (0.12-12.75) | 0.862 |
| *HLA-DPB1 *0101* | 0.05 | 0.09 | 0.54 | (0.26-1.11) | 0.092 |
| *HLA-DPB1 *0201* | 0.26 | 0.15 | 2.03 | (1.35-3.03) | 5.91E04 |
| *HLA-DPB1 *0202* | 0.01 | 0.01 | 0.33 | (0.02-6.2) | 0.457 |
| *HLA-DPB1 *0301* | 0.07 | 0.11 | 0.61 | (0.33-1.16) | 0.132 |
| *HLA-DPB1 *0401* | 0.32 | 0.41 | 0.67 | (0.47-0.95) | 0.024 |
| *HLA-DPB1 *0402* | 0.11 | 0.11 | 1.03 | (0.62-1.71) | 0.911 |
| *HLA-DPB1 *0601* | 0.01 | 0.02 | 0.1 | (0-2.48) | 0.159 |
| *HLA-DPB1 *0901* | 0.01 | 0.01 | 1.3 | (0.24-7.2) | 0.763 |
| *HLA-DPB1 *1001* | 0.02 | 0.02 | 1.19 | (0.32-4.4) | 0.794 |
| *HLA-DPB1 *1101* | 0.02 | 0.02 | 1.08 | (0.3-3.94) | 0.906 |
| *HLA-DPB1 *1301* | 0.01 | 0.01 | 0.37 | (0.04-3.16) | 0.364 |
| *HLA-DPB1 *1401* | 0.03 | 0.01 | 2.44 | (0.79-7.58) | 0.122 |
| *HLA-DPB1 *1501* | 0.01 | 0.01 | 1.57 | (0.29-8.67) | 0.602 |
| *HLA-DPB1 *1601* | 0.01 | 0.01 | 1.31 | (0.26-6.69) | 0.744 |
| *HLA-DPB1 *1701* | 0.01 | 0.01 | 0.44 | (0.06-3.38) | 0.433 |
| *HLA-DPB1 *1901* | 0.01 | 0.01 | 1.05 | (0.22-5.06) | 0.949 |
| *HLA-DPB1 *2301* | 0.01 | 0.01 | 2.44 | (0.23-25.84) | 0.460 |
| *HLA-DQB1 *0201* | 0.1 | 0.15 | 0.61 | (0.36-1.02) | 0.061 |
| *HLA-DQB1 *0202* | 0.02 | 0.08 | 0.19 | (0.06-0.61) | 5.87E03 |
| *HLA-DQB1 *0301* | 0.26 | 0.15 | 1.91 | (1.31-2.78) | 7.45E04 |
| *HLA-DQB1 *0302* | 0.02 | 0.11 | 0.17 | (0.06-0.49) | 8.49E04 |
| *HLA-DQB1 *0303* | 0.04 | 0.05 | 0.86 | (0.38-1.93) | 0.707 |
| *HLA-DQB1 *0402* | 0.16 | 0.02 | 10.11 | (5.32-19.22) | 1.69E-12 |
| *HLA-DQB1 *0501* | 0.08 | 0.11 | 0.71 | (0.4-1.26) | 0.242 |
| *HLA-DQB1 *0502* | 0.05 | 0.02 | 2.82 | (1.21-6.6) | 0.017 |
| *HLA-DQB1 *0503* | 0.02 | 0.03 | 1 | (0.33-2.99) | 0.995 |
| *HLA-DQB1 *0602* | 0.05 | 0.13 | 0.39 | (0.2-0.75) | 0.005 |
| *HLA-DQB1 *0603* | 0.14 | 0.07 | 2.26 | (1.36-3.76) | 0.002 |
| *HLA-DQB1 *0604* | 0.05 | 0.08 | 0.66 | (0.33-1.34) | 0.253 |
| *HLA-DRB1 *0101* | 0.08 | 0.04 | 2.73 | (1.33-5.63) | 0.006 |

Supplementary table 4. (continued)

| Classical Allele | Frequency | | OR | 95% CI | P-value |
|---|---|---|---|---|---|
| | Cases | Controls | | | |
| *HLA-DRB1 *0102* | 0.01 | 0.01 | 0.44 | (0.05-3.6) | 0.446 |
| *HLA-DRB1 *0301* | 0.09 | 0.14 | 0.62 | (0.36-1.06) | 0.081 |
| *HLA-DRB1 *0401* | 0.02 | 0.09 | 0.15 | (0.04-0.51) | 0.003 |
| *HLA-DRB1 *0701* | 0.02 | 0.11 | 0.12 | (0.04-0.41) | 6.58E04 |
| *HLA-DRB1 *0801* | 0.16 | 0.02 | 11.12 | (5.76-21.43) | 6.51E-13 |
| *HLA-DRB1 *0901* | 0.04 | 0.02 | 2.06 | (0.88-4.81) | 0.096 |
| *HLA-DRB1 *1101* | 0.09 | 0.06 | 1.63 | (0.87-3.06) | 0.125 |
| *HLA-DRB1 *1104* | 0.1 | 0.02 | 5.63 | (2.79-11.36) | 1.37E06 |
| *HLA-DRB1 *1201* | 0.03 | 0.01 | 1.95 | (0.65-5.83) | 0.230 |
| *HLA-DRB1 *1301* | 0.12 | 0.09 | 1.55 | (0.93-2.59) | 0.094 |
| *HLA-DRB1 *1302* | 0.06 | 0.07 | 0.87 | (0.44-1.72) | 0.689 |
| *HLA-DRB1 *1303* | 0.03 | 0.01 | 2.33 | (0.75-7.25) | 0.145 |
| *HLA-DRB1 *1404* | 0.01 | 0.01 | 1.12 | (0.04-28.14) | 0.945 |
| *HLA-DRB1 *1501* | 0.06 | 0.14 | 0.42 | (0.22-0.8) | 0.008 |
| *HLA-DRB1 *1601* | 0.02 | 0.02 | 1.22 | (0.38-3.86) | 0.741 |
| **Acute anterior uveitis (n=18)** | | | | | |
| *HLA-A *0101* | 0.11 | 0.2 | | | |
| *HLA-A *0201* | 0.33 | 0.29 | | | |
| *HLA-A *0301* | 0.08 | 0.16 | | | |
| *HLA-A *1101* | 0.08 | 0.05 | | | |
| HLA-A *2301 | 0.03 | 0.02 | | | |
| *HLA-A *2402* | 0.11 | 0.08 | | | |
| *HLA-A *2902* | 0.03 | 0.02 | | | |
| *HLA-A *3201* | 0.03 | 0.04 | | | |
| *HLA-A *6801* | 0.08 | 0.03 | | | |
| HLA-B *0702 | 0.06 | 0.14 | | | |
| *HLA-B *0801* | 0.08 | 0.14 | | | |
| *HLA-B *1501* | 0.11 | 0.09 | | | |
| *HLA-B *1801* | 0.03 | 0.03 | | | |
| *HLA-B *2705* | 0.17 | 0.03 | | | |
| HLA-B *3501 | 0.08 | 0.07 | | | |
| *HLA-B *3503* | 0.03 | 0.02 | | | |
| *HLA-B *3701* | 0.03 | 0.02 | | | |
| *HLA-B *4001* | 0.03 | 0.06 | | | |
| *HLA-B *4402* | 0.06 | 0.08 | | | |
| HLA-B *4403 | 0.03 | 0.05 | | | |
| *HLA-B *4901* | 0.03 | 0.01 | | | |
| *HLA-B *5101* | 0.03 | 0.06 | | | |
| *HLA-B *5501* | 0.03 | 0.02 | | | |
| *HLA-B *5701* | 0.06 | 0.03 | | | |
| HLA-B *5801 | 0.03 | 0.01 | | | |
| *HLA-C *0102* | 0.03 | 0.03 | | | |
| *HLA-C *0202* | 0.08 | 0.05 | | | |
| *HLA-C *0302* | 0.03 | 0.01 | | | |

Supplementary table 4. (continued)

| Classical Allele | Frequency | | OR | 95% CI | P-value |
|---|---|---|---|---|---|
| | Cases | Controls | | | |
| *HLA-C *0303* | 0.17 | 0.07 | | | |
| HLA-C *0304 | 0.03 | 0.11 | | | |
| *HLA-C *0401* | 0.14 | 0.11 | | | |
| *HLA-C *0501* | 0.06 | 0.07 | | | |
| *HLA-C *0602* | 0.08 | 0.09 | | | |
| *HLA-C *0701* | 0.11 | 0.17 | | | |
| HLA-C *0702 | 0.08 | 0.16 | | | |
| *HLA-C *0704* | 0.06 | 0.02 | | | |
| *HLA-C *1402* | 0.06 | 0.01 | | | |
| *HLA-C *1601* | 0.06 | 0.04 | | | |
| *HLA-DPB1 *0101* | 0.06 | 0.09 | | | |
| HLA-DPB1 *0201 | 0.22 | 0.15 | | | |
| *HLA-DPB1 *0202* | 0.03 | 0.01 | | | |
| *HLA-DPB1 *0301* | 0.14 | 0.11 | | | |
| *HLA-DPB1 *0401* | 0.25 | 0.41 | | | |
| *HLA-DPB1 *0402* | 0.11 | 0.11 | | | |
| HLA-DPB1 *0501 | 0.06 | 0.01 | | | |
| *HLA-DPB1 *1401* | 0.03 | 0.01 | | | |
| *HLA-DPB1 *1701* | 0.03 | 0.01 | | | |
| *HLA-DQB1 *0201* | 0.08 | 0.15 | | | |
| *HLA-DQB1 *0202* | 0.08 | 0.08 | | | |
| HLA-DQB1 *0301 | 0.08 | 0.15 | | | |
| *HLA-DQB1 *0302* | 0.08 | 0.11 | | | |
| *HLA-DQB1 *0303* | 0.08 | 0.05 | | | |
| *HLA-DQB1 *0402* | 0.08 | 0.02 | | | |
| *HLA-DQB1 *0501* | 0.14 | 0.11 | | | |
| HLA-DQB1 *0502 | 0.06 | 0.02 | | | |
| *HLA-DQB1 *0503* | 0.03 | 0.03 | | | |
| *HLA-DQB1 *0602* | 0.11 | 0.13 | | | |
| *HLA-DQB1 *0603* | 0.11 | 0.07 | | | |
| *HLA-DQB1 *0604* | 0.03 | 0.08 | | | |
| HLA-DQB1 *0609 | 0.03 | 0.01 | | | |
| *HLA-DRB1 *0101* | 0.06 | 0.04 | | | |
| *HLA-DRB1 *0102* | 0.03 | 0.01 | | | |
| *HLA-DRB1 *0103* | 0.03 | 0.05 | | | |
| *HLA-DRB1 *0301* | 0.08 | 0.14 | | | |
| HLA-DRB1 *0401 | 0.06 | 0.09 | | | |
| *HLA-DRB1 *0403* | 0.03 | 0.01 | | | |
| *HLA-DRB1 *0701* | 0.17 | 0.11 | | | |
| *HLA-DRB1 *0801* | 0.08 | 0.02 | | | |
| *HLA-DRB1 *1101* | 0.06 | 0.06 | | | |
| HLA-DRB1 *1301 | 0.11 | 0.09 | | | |
| *HLA-DRB1 *1302* | 0.06 | 0.07 | | | |
| *HLA-DRB1 *1303* | 0.03 | 0.01 | | | |

Supplementary table 4. (continued)

| Classical Allele | Frequency | | OR | 95% CI | P-value |
|---|---|---|---|---|---|
| | Cases | Controls | | | |
| *HLA-DRB1 *1501* | 0.14 | 0.14 | | | |
| **Idiopathic chronic anterior uveitis. no JIA (n=50)** | | | | | |
| *HLA-A *0101* | 0.16 | 0.2 | 0.75 | (0.42-1.33) | 0.323 |
| *HLA-A *0201* | 0.33 | 0.29 | 1.24 | (0.8-1.94) | 0.333 |
| *HLA-A *0205* | 0.01 | 0.01 | 2.18 | (0.22-21.77) | 0.508 |
| *HLA-A *0301* | 0.09 | 0.16 | 0.5 | (0.24-1.02) | 0.057 |
| *HLA-A *1101* | 0.07 | 0.05 | 1.53 | (0.64-3.62) | 0.338 |
| *HLA-A *2402* | 0.12 | 0.08 | 1.62 | (0.84-3.11) | 0.151 |
| *HLA-A *2601* | 0.08 | 0.03 | 3.19 | (1.33-7.68) | 0.009 |
| *HLA-A *3001* | 0.02 | 0.01 | 1.55 | (0.32-7.38) | 0.584 |
| *HLA-A *3101* | 0.01 | 0.04 | 0.25 | (0.03-1.92) | 0.181 |
| *HLA-A *3201* | 0.02 | 0.04 | 0.33 | (0.06-1.77) | 0.196 |
| *HLA-A *6801* | 0.02 | 0.03 | 0.58 | (0.13-2.55) | 0.473 |
| *HLA-B *0702* | 0.06 | 0.14 | 0.39 | (0.16-0.91) | 0.030 |
| *HLA-B *0801* | 0.16 | 0.14 | 1.19 | (0.67-2.13) | 0.546 |
| *HLA-B *1302* | 0.01 | 0.02 | 0.45 | (0.06-3.41) | 0.437 |
| *HLA-B *1402* | 0.03 | 0.02 | 1.67 | (0.5-5.63) | 0.405 |
| *HLA-B *1501* | 0.07 | 0.09 | 0.71 | (0.32-1.58) | 0.404 |
| *HLA-B *1517* | 0.01 | 0.01 | 2.29 | (0.25-20.53) | 0.460 |
| *HLA-B *1801* | 0.06 | 0.03 | 2.03 | (0.84-4.86) | 0.114 |
| *HLA-B *2705* | 0.03 | 0.03 | 1.34 | (0.38-4.76) | 0.647 |
| *HLA-B *3501* | 0.09 | 0.07 | 1.46 | (0.68-3.13) | 0.328 |
| *HLA-B *3503* | 0.02 | 0.02 | 1.36 | (0.27-6.86) | 0.707 |
| *HLA-B *3701* | 0.01 | 0.02 | 0.47 | (0.06-3.74) | 0.479 |
| *HLA-B *3801* | 0.05 | 0.01 | 5.67 | (1.79-17.95) | 0.003 |
| *HLA-B *3901* | 0.01 | 0.01 | 0.72 | (0.08-6.58) | 0.774 |
| *HLA-B *4001* | 0.06 | 0.06 | 0.88 | (0.38-2.06) | 0.770 |
| *HLA-B *4002* | 0.02 | 0.02 | 1.08 | (0.23-4.93) | 0.926 |
| *HLA-B *4402* | 0.11 | 0.08 | 1.39 | (0.7-2.75) | 0.348 |
| *HLA-B *4403* | 0.01 | 0.05 | 0.2 | (0.03-1.47) | 0.113 |
| *HLA-B *5001* | 0.02 | 0.01 | 2.43 | (0.47-12.57) | 0.289 |
| *HLA-B *5101* | 0.04 | 0.06 | 0.63 | (0.22-1.83) | 0.393 |
| *HLA-B *5501* | 0.01 | 0.02 | 0.49 | (0.06-3.94) | 0.501 |
| *HLA-C *0102* | 0.03 | 0.03 | 1.16 | (0.33-4.08) | 0.822 |
| *HLA-C *0202* | 0.06 | 0.05 | 1.37 | (0.56-3.35) | 0.496 |
| *HLA-C *0303* | 0.08 | 0.07 | 1.07 | (0.49-2.33) | 0.875 |
| *HLA-C *0304* | 0.07 | 0.11 | 0.54 | (0.24-1.22) | 0.140 |
| *HLA-C *0401* | 0.14 | 0.11 | 1.35 | (0.75-2.44) | 0.313 |
| *HLA-C *0501* | 0.09 | 0.07 | 1.42 | (0.66-3.04) | 0.366 |
| *HLA-C *0602* | 0.04 | 0.09 | 0.46 | (0.16-1.29) | 0.139 |
| *HLA-C *0701* | 0.2 | 0.17 | 1.26 | (0.74-2.15) | 0.388 |
| *HLA-C *0702* | 0.08 | 0.16 | 0.46 | (0.22-0.97) | 0.042 |
| *HLA-C *0704* | 0.01 | 0.02 | 0.43 | (0.05-3.51) | 0.428 |
| *HLA-C *0802* | 0.02 | 0.02 | 0.83 | (0.18-3.76) | 0.813 |

Supplementary table 4. (continued)

| Classical Allele | Frequency | | OR | 95% CI | P-value |
|---|---|---|---|---|---|
| | Cases | Controls | | | |
| *HLA-C *1203* | 0.08 | 0.03 | 2.75 | (1.24-6.1) | 0.013 |
| *HLA-C *1402* | 0.01 | 0.01 | 0.83 | (0.1-7.01) | 0.860 |
| *HLA-C *1502* | 0.02 | 0.02 | 0.74 | (0.16-3.44) | 0.701 |
| *HLA-C *1602* | 0.02 | 0.01 | 3.38 | (0.62-18.48) | 0.159 |
| *HLA-C *1701* | 0.02 | 0.01 | 4.84 | (0.83-28.12) | 0.079 |
| *HLA-DPB1 *0101* | 0.07 | 0.09 | 0.8 | (0.36-1.8) | 0.592 |
| *HLA-DPB1 *0201* | 0.2 | 0.15 | 1.4 | (0.82-2.41) | 0.221 |
| *HLA-DPB1 *0301* | 0.1 | 0.11 | 0.93 | (0.45-1.9) | 0.833 |
| *HLA-DPB1 *0401* | 0.32 | 0.41 | 0.7 | (0.44-1.09) | 0.111 |
| *HLA-DPB1 *0402* | 0.11 | 0.11 | 0.97 | (0.5-1.88) | 0.926 |
| *HLA-DPB1 *0501* | 0.02 | 0.01 | 1.61 | (0.34-7.7) | 0.551 |
| *HLA-DPB1 *0601* | 0.01 | 0.02 | 0.34 | (0.02-5.78) | 0.458 |
| *HLA-DPB1 *0901* | 0.02 | 0.01 | 2.65 | (0.48-14.52) | 0.262 |
| *HLA-DPB1 *1101* | 0.01 | 0.02 | 0.59 | (0.07-4.87) | 0.627 |
| *HLA-DPB1 *1301* | 0.02 | 0.01 | 1.62 | (0.34-7.68) | 0.543 |
| *HLA-DPB1 *1401* | 0.03 | 0.01 | 2.43 | (0.62-9.56) | 0.202 |
| *HLA-DPB1 *1601* | 0.01 | 0.01 | 1.22 | (0.14-10.82) | 0.857 |
| *HLA-DPB1 *1701* | 0.01 | 0.01 | 0.75 | (0.1-5.38) | 0.773 |
| *HLA-DPB1 *1901* | 0.01 | 0.01 | 0.94 | (0.11-7.93) | 0.954 |
| *HLA-DPB1 *2301* | 0.01 | 0.01 | 2.47 | (0.08-77.52) | 0.607 |
| *HLA-DQB1 *0201* | 0.17 | 0.15 | 1.17 | (0.66-2.06) | 0.589 |
| *HLA-DQB1 *0202* | 0.04 | 0.08 | 0.48 | (0.17-1.37) | 0.171 |
| *HLA-DQB1 *0301* | 0.18 | 0.15 | 1.17 | (0.7-1.97) | 0.546 |
| *HLA-DQB1 *0302* | 0.07 | 0.11 | 0.61 | (0.27-1.36) | 0.226 |
| *HLA-DQB1 *0303* | 0.04 | 0.05 | 0.92 | (0.33-2.58) | 0.870 |
| *HLA-DQB1 *0402* | 0.1 | 0.02 | 5.37 | (2.38-12.09) | 5.07E-05 |
| *HLA-DQB1 *0501* | 0.06 | 0.11 | 0.5 | (0.21-1.19) | 0.118 |
| *HLA-DQB1 *0502* | 0.01 | 0.02 | 0.51 | (0.07-3.99) | 0.524 |
| *HLA-DQB1 *0503* | 0.1 | 0.03 | 5.10 | (2.25-11.58) | 9.73E-05 |
| *HLA-DQB1 *0602* | 0.04 | 0.13 | 0.28 | (0.1-0.77) | 0.014 |
| *HLA-DQB1 *0603* | 0.14 | 0.07 | 2.19 | (1.17-4.07) | 0.014 |
| *HLA-DQB1 *0604* | 0.04 | 0.08 | 0.47 | (0.16-1.36) | 0.166 |
| *HLA-DRB1 *0101* | 0.02 | 0.04 | 0.43 | (0.07-2.53) | 0.353 |
| *HLA-DRB1 *0102* | 0.03 | 0.01 | 2.37 | (0.7-8.04) | 0.167 |
| *HLA-DRB1 *0301* | 0.17 | 0.14 | 1.27 | (0.72-2.23) | 0.414 |
| *HLA-DRB1 *0401* | 0.01 | 0.09 | 0.08 | (0.01-0.72) | 0.024 |
| *HLA-DRB1 *0403* | 0.02 | 0.01 | 5.05 | (0.65-39.2) | 0.122 |
| *HLA-DRB1 *0404* | 0.02 | 0.03 | 0.5 | (0.1-2.45) | 0.394 |
| *HLA-DRB1 *0701* | 0.06 | 0.11 | 0.51 | (0.21-1.22) | 0.129 |
| *HLA-DRB1 *0801* | 0.09 | 0.02 | 5.30 | (2.26-12.44) | 1.30E-04 |
| *HLA-DRB1 *0901* | 0.02 | 0.02 | 1.04 | (0.25-4.32) | 0.962 |
| *HLA-DRB1 *1101* | 0.08 | 0.06 | 1.39 | (0.61-3.18) | 0.436 |
| *HLA-DRB1 *1104* | 0.04 | 0.02 | 2.05 | (0.7-6.02) | 0.189 |
| *HLA-DRB1 *1201* | 0.03 | 0.01 | 2.09 | (0.55-7.89) | 0.277 |

Supplementary table 4. (continued)

| Classical Allele | Frequency | | OR | 95% CI | P-value |
|---|---|---|---|---|---|
| | Cases | Controls | | | |
| *HLA-DRB1 *1301* | 0.14 | 0.09 | 1.78 | (0.96-3.3) | 0.068 |
| *HLA-DRB1 *1302* | 0.04 | 0.07 | 0.56 | (0.19-1.61) | 0.283 |
| *HLA-DRB1 *1401* | 0.02 | 0.03 | 0.93 | (0.18-4.81) | 0.928 |
| *HLA-DRB1 *1404* | 0.02 | 0.01 | 10.27 | (1.01-104.96) | 0.049 |
| *HLA-DRB1 *1501* | 0.04 | 0.14 | 0.27 | (0.1-0.76) | 0.013 |
| **Intermediate uveitis (n=39)** | | | | | |
| *HLA-A *0101* | 0.18 | 0.2 | 0.86 | (0.46-1.6) | 0.635 |
| *HLA-A *0201* | 0.17 | 0.29 | 0.49 | (0.27-0.9) | 0.021 |
| *HLA-A *0301* | 0.19 | 0.16 | 1.29 | (0.71-2.35) | 0.396 |
| *HLA-A *1101* | 0.04 | 0.05 | 0.8 | (0.24-2.74) | 0.729 |
| *HLA-A *2402* | 0.06 | 0.08 | 0.79 | (0.31-2.05) | 0.633 |
| *HLA-A *2601* | 0.05 | 0.03 | 1.68 | (0.54-5.25) | 0.369 |
| *HLA-A *2902* | 0.03 | 0.02 | 1.5 | (0.31-7.22) | 0.612 |
| *HLA-A *3001* | 0.04 | 0.01 | 3.27 | (0.87-12.26) | 0.079 |
| *HLA-A *3101* | 0.04 | 0.04 | 1.09 | (0.33-3.63) | 0.888 |
| *HLA-A *3201* | 0.04 | 0.04 | 0.87 | (0.23-3.37) | 0.842 |
| *HLA-A *6801* | 0.06 | 0.03 | 1.85 | (0.72-4.72) | 0.198 |
| *HLA-B *0702* | 0.15 | 0.14 | 1.13 | (0.58-2.21) | 0.722 |
| *HLA-B *0801* | 0.14 | 0.14 | 1.01 | (0.52-1.98) | 0.970 |
| *HLA-B *1302* | 0.01 | 0.02 | 0.53 | (0.07-4.07) | 0.543 |
| *HLA-B *1402* | 0.01 | 0.02 | 0.73 | (0.1-5.3) | 0.759 |
| *HLA-B *1501* | 0.08 | 0.09 | 0.85 | (0.37-1.98) | 0.709 |
| *HLA-B *1801* | 0.04 | 0.03 | 1.21 | (0.38-3.82) | 0.745 |
| *HLA-B *2705* | 0.04 | 0.03 | 1.51 | (0.43-5.37) | 0.521 |
| *HLA-B *3501* | 0.05 | 0.07 | 0.76 | (0.26-2.21) | 0.610 |
| *HLA-B *3701* | 0.03 | 0.02 | 1.27 | (0.28-5.69) | 0.755 |
| *HLA-B *3801* | 0.01 | 0.01 | 0.94 | (0.11-8.4) | 0.956 |
| *HLA-B *3901* | 0.03 | 0.01 | 2.4 | (0.49-11.89) | 0.282 |
| *HLA-B *4001* | 0.03 | 0.06 | 0.4 | (0.1-1.64) | 0.201 |
| *HLA-B *4002* | 0.01 | 0.02 | 0.68 | (0.08-5.51) | 0.715 |
| *HLA-B *4402* | 0.06 | 0.08 | 0.77 | (0.3-1.99) | 0.585 |
| *HLA-B *4403* | 0.04 | 0.05 | 0.75 | (0.23-2.44) | 0.632 |
| *HLA-B *4901* | 0.01 | 0.01 | 1.84 | (0.22-15.42) | 0.575 |
| *HLA-B *5101* | 0.12 | 0.06 | 2.41 | (1.1-5.3) | 0.029 |
| *HLA-B *5501* | 0.03 | 0.02 | 1.36 | (0.3-6.21) | 0.689 |
| *HLA-B *5701* | 0.03 | 0.03 | 0.8 | (0.18-3.5) | 0.769 |
| *HLA-C *0102* | 0.01 | 0.03 | 0.45 | (0.06-3.66) | 0.454 |
| *HLA-C *0202* | 0.05 | 0.05 | 1.12 | (0.39-3.22) | 0.839 |
| *HLA-C *0303* | 0.09 | 0.07 | 1.27 | (0.55-2.94) | 0.580 |
| *HLA-C *0304* | 0.04 | 0.11 | 0.31 | (0.09-1.02) | 0.055 |
| *HLA-C *0401* | 0.08 | 0.11 | 0.68 | (0.29-1.59) | 0.373 |
| *HLA-C *0501* | 0.05 | 0.07 | 0.75 | (0.26-2.17) | 0.590 |
| *HLA-C *0602* | 0.08 | 0.09 | 0.85 | (0.36-2.04) | 0.721 |
| *HLA-C *0701* | 0.19 | 0.17 | 1.16 | (0.64-2.09) | 0.629 |

Supplementary table 4. (continued)

| Classical Allele | Frequency | | OR | 95% CI | P-value |
|---|---|---|---|---|---|
| | Cases | Controls | | | |
| *HLA-C *0702* | 0.15 | 0.16 | 0.96 | (0.5-1.85) | 0.913 |
| *HLA-C *0704* | 0.04 | 0.02 | 2.08 | (0.57-7.51) | 0.266 |
| *HLA-C *0802* | 0.01 | 0.02 | 0.48 | (0.06-3.94) | 0.497 |
| *HLA-C *1203* | 0.03 | 0.03 | 0.81 | (0.19-3.46) | 0.778 |
| *HLA-C *1402* | 0.03 | 0.01 | 2.3 | (0.48-10.99) | 0.297 |
| *HLA-C *1502* | 0.08 | 0.02 | 3.84 | (1.43-10.34) | 0.008 |
| *HLA-C *1601* | 0.01 | 0.04 | 0.29 | (0.04-2.31) | 0.240 |
| *HLA-C *1701* | 0.01 | 0.01 | 2.95 | (0.3-28.71) | 0.351 |
| *HLA-DPB1 *0101* | 0.04 | 0.09 | 0.42 | (0.13-1.38) | 0.153 |
| *HLA-DPB1 *0201* | 0.1 | 0.15 | 0.63 | (0.29-1.39) | 0.253 |
| *HLA-DPB1 *0301* | 0.13 | 0.11 | 1.27 | (0.61-2.64) | 0.525 |
| *HLA-DPB1 *0401* | 0.5 | 0.41 | 1.5 | (0.93-2.42) | 0.097 |
| *HLA-DPB1 *0402* | 0.08 | 0.11 | 0.65 | (0.27-1.55) | 0.333 |
| *HLA-DPB1 *0501* | 0.01 | 0.01 | 1.07 | (0.13-8.9) | 0.952 |
| *HLA-DPB1 *0601* | 0.01 | 0.02 | 0.54 | (0.03-8.61) | 0.663 |
| *HLA-DPB1 *0901* | 0.03 | 0.01 | 3.16 | (0.58-17.14) | 0.182 |
| *HLA-DPB1 *1301* | 0.01 | 0.01 | 0.87 | (0.11-7.13) | 0.893 |
| *HLA-DPB1 *1401* | 0.01 | 0.01 | 0.99 | (0.11-9.17) | 0.994 |
| *HLA-DPB1 *1601* | 0.01 | 0.01 | 1.57 | (0.18-13.65) | 0.683 |
| *HLA-DPB1 *1701* | 0.01 | 0.01 | 0.94 | (0.13-6.83) | 0.955 |
| *HLA-DPB1 *2001* | 0.01 | 0.01 | 5.68 | (0.29-110.5) | 0.252 |
| *HLA-DQB1 *0201* | 0.14 | 0.15 | 0.96 | (0.49-1.89) | 0.910 |
| *HLA-DQB1 *0202* | 0.03 | 0.08 | 0.29 | (0.07-1.23) | 0.094 |
| *HLA-DQB1 *0301* | 0.14 | 0.15 | 0.92 | (0.48-1.76) | 0.805 |
| *HLA-DQB1 *0302* | 0.12 | 0.11 | 1.11 | (0.53-2.34) | 0.780 |
| *HLA-DQB1 *0303* | 0.03 | 0.05 | 0.55 | (0.13-2.3) | 0.409 |
| *HLA-DQB1 *0402* | 0.04 | 0.02 | 1.93 | (0.59-6.32) | 0.277 |
| *HLA-DQB1 *0501* | 0.09 | 0.11 | 0.77 | (0.35-1.74) | 0.536 |
| *HLA-DQB1 *0502* | 0.03 | 0.02 | 1.46 | (0.32-6.57) | 0.624 |
| *HLA-DQB1 *0503* | 0.01 | 0.03 | 0.43 | (0.06-3.34) | 0.420 |
| *HLA-DQB1 *0602* | 0.23 | 0.13 | 1.92 | (1.1-3.35) | 0.022 |
| *HLA-DQB1 *0603* | 0.09 | 0.07 | 1.33 | (0.59-3) | 0.484 |
| *HLA-DQB1 *0604* | 0.05 | 0.08 | 0.62 | (0.21-1.8) | 0.382 |
| *HLA-DRB1 *0101* | 0.06 | 0.04 | 2.1 | (0.69-6.37) | 0.189 |
| *HLA-DRB1 *0102* | 0.03 | 0.01 | 2.02 | (0.5-8.25) | 0.326 |
| *HLA-DRB1 *0103* | 0.01 | 0.05 | 0.16 | (0.02-1.72) | 0.131 |
| *HLA-DRB1 *0301* | 0.14 | 0.14 | 1.04 | (0.53-2.04) | 0.909 |
| *HLA-DRB1 *0401* | 0.05 | 0.09 | 0.55 | (0.19-1.61) | 0.274 |
| *HLA-DRB1 *0403* | 0.03 | 0.01 | 8.98 | (1.16-69.32) | 0.035 |
| *HLA-DRB1 *0404* | 0.03 | 0.03 | 0.71 | (0.15-3.41) | 0.667 |
| *HLA-DRB1 *0407* | 0.04 | 0.01 | 4.1 | (1.05-15.97) | 0.042 |
| *HLA-DRB1 *0701* | 0.04 | 0.11 | 0.29 | (0.09-0.96) | 0.043 |
| *HLA-DRB1 *0801* | 0.03 | 0.02 | 1.45 | (0.34-6.2) | 0.617 |
| *HLA-DRB1 *0901* | 0.01 | 0.02 | 0.72 | (0.1-5.39) | 0.753 |

Supplementary table 4. (continued)

| Classical Allele | Frequency | | OR | 95% CI | P-value |
|---|---|---|---|---|---|
| | Cases | Controls | | | |
| *HLA-DRB1 *1101* | 0.05 | 0.06 | 0.86 | (0.28-2.64) | 0.794 |
| *HLA-DRB1 *1104* | 0.01 | 0.02 | 0.55 | (0.06-5.07) | 0.597 |
| *HLA-DRB1 *1301* | 0.05 | 0.09 | 0.57 | (0.2-1.61) | 0.286 |
| *HLA-DRB1 *1302* | 0.05 | 0.07 | 0.77 | (0.27-2.21) | 0.624 |
| *HLA-DRB1 *1303* | 0.01 | 0.01 | 1.15 | (0.13-10.22) | 0.902 |
| *HLA-DRB1 *1501* | 0.26 | 0.14 | 2.18 | (1.26-3.77) | 0.005 |
| *HLA-DRB1 *1601* | 0.03 | 0.02 | 1.69 | (0.36-7.95) | 0.504 |
| **Panuveitis (n=81)** | | | | | |
| *HLA-A *0101* | 0.18 | 0.2 | 0.86 | (0.56 -1.33) | 0.503 |
| *HLA-A *0201* | 0.2 | 0.29 | 0.62 | (0.42 -0.93) | 0.022 |
| *HLA-A *0205* | 0.03 | 0.01 | 6.68 | (1.83 -24.34) | 0.004 |
| *HLA-A *0301* | 0.14 | 0.16 | 0.89 | (0.55 -1.44) | 0.640 |
| *HLA-A *1101* | 0.06 | 0.05 | 1.21 | (0.58 -2.55) | 0.612 |
| *HLA-A *2301* | 0.02 | 0.02 | 0.89 | (0.27 -2.96) | 0.845 |
| *HLA-A *2402* | 0.09 | 0.08 | 1.19 | (0.66 -2.13) | 0.556 |
| *HLA-A *2601* | 0.01 | 0.03 | 0.32 | (0.07 -1.49) | 0.147 |
| *HLA-A *2902* | 0.02 | 0.02 | 1.04 | (0.28 -3.86) | 0.951 |
| *HLA-A *3001* | 0.01 | 0.01 | 0.44 | (0.05 -3.82) | 0.458 |
| *HLA-A *3101* | 0.02 | 0.04 | 0.7 | (0.25 -1.96) | 0.492 |
| *HLA-A *3201* | 0.02 | 0.04 | 0.48 | (0.15 -1.58) | 0.226 |
| *HLA-A *6801* | 0.06 | 0.03 | 1.86 | (0.89 -3.86) | 0.098 |
| *HLA-B *0702* | 0.04 | 0.14 | 0.22 | (0.1 -0.52) | 0.001 |
| *HLA-B *0801* | 0.11 | 0.14 | 0.77 | (0.46 -1.3) | 0.333 |
| *HLA-B *1402* | 0.04 | 0.02 | 2.4 | (1 -5.76) | 0.051 |
| *HLA-B *1501* | 0.05 | 0.09 | 0.53 | (0.25 -1.11) | 0.093 |
| *HLA-B *1517* | 0.01 | 0.01 | 2.4 | (0.45 -12.71) | 0.303 |
| *HLA-B *1801* | 0.04 | 0.03 | 1.35 | (0.6 -3.03) | 0.464 |
| *HLA-B *2705* | 0.03 | 0.03 | 1.18 | (0.43 -3.24) | 0.744 |
| *HLA-B *3501* | 0.09 | 0.07 | 1.48 | (0.82 -2.66) | 0.197 |
| *HLA-B *3503* | 0.01 | 0.02 | 0.7 | (0.14 -3.52) | 0.661 |
| *HLA-B *3701* | 0.02 | 0.02 | 0.9 | (0.26 -3.13) | 0.870 |
| *HLA-B *3801* | 0.02 | 0.01 | 2.03 | (0.61 -6.77) | 0.248 |
| *HLA-B *3901* | 0.02 | 0.01 | 1.61 | (0.42 -6.16) | 0.487 |
| *HLA-B *3906* | 0.01 | 0.01 | 0.72 | (0.08 -6.11) | 0.759 |
| *HLA-B *4001* | 0.04 | 0.06 | 0.67 | (0.3 -1.48) | 0.320 |
| *HLA-B *4402* | 0.09 | 0.08 | 1.06 | (0.58 -1.94) | 0.842 |
| *HLA-B *4403* | 0.04 | 0.05 | 0.85 | (0.38 -1.89) | 0.683 |
| *HLA-B *4901* | 0.02 | 0.01 | 2.66 | (0.67 -10.53) | 0.164 |
| *HLA-B *5001* | 0.01 | 0.01 | 0.58 | (0.06 -5.6) | 0.636 |
| *HLA-B *5101* | 0.07 | 0.06 | 1.41 | (0.72 -2.77) | 0.315 |
| *HLA-B *5501* | 0.01 | 0.02 | 0.63 | (0.14 -2.82) | 0.542 |
| *HLA-B *5601* | 0.01 | 0.01 | 1.84 | (0.36 -9.45) | 0.467 |
| *HLA-B *5701* | 0.01 | 0.03 | 0.37 | (0.09 -1.6) | 0.184 |
| *HLA-B *5801* | 0.01 | 0.01 | 1.83 | (0.36 -9.18) | 0.464 |

Supplementary table 4. (continued)

| Classical Allele | Frequency | | OR | 95% CI | P-value |
|---|---|---|---|---|---|
| | Cases | Controls | | | |
| *HLA-C *0102* | 0.03 | 0.03 | 1.17 | (0.44 -3.09) | 0.748 |
| *HLA-C *0202* | 0.04 | 0.05 | 0.93 | (0.41 -2.13) | 0.872 |
| *HLA-C *0303* | 0.06 | 0.07 | 0.73 | (0.35 -1.52) | 0.397 |
| *HLA-C *0304* | 0.06 | 0.11 | 0.51 | (0.26 -1.02) | 0.059 |
| *HLA-C *0401* | 0.14 | 0.11 | 1.27 | (0.78 -2.07) | 0.328 |
| *HLA-C *0501* | 0.07 | 0.07 | 1.01 | (0.52 -1.98) | 0.972 |
| *HLA-C *0602* | 0.06 | 0.09 | 0.6 | (0.3 -1.23) | 0.165 |
| *HLA-C *0701* | 0.15 | 0.17 | 0.89 | (0.56 -1.4) | 0.602 |
| *HLA-C *0702* | 0.09 | 0.16 | 0.53 | (0.3 -0.94) | 0.030 |
| *HLA-C *0704* | 0.02 | 0.02 | 1.26 | (0.41 -3.89) | 0.683 |
| *HLA-C *0802* | 0.04 | 0.02 | 1.76 | (0.75 -4.17) | 0.196 |
| *HLA-C *1203* | 0.04 | 0.03 | 1.4 | (0.6 -3.22) | 0.435 |
| *HLA-C *1402* | 0.04 | 0.01 | 3.39 | (1.21 -9.49) | 0.020 |
| *HLA-C *1502* | 0.04 | 0.02 | 1.6 | (0.61 -4.16) | 0.336 |
| *HLA-C *1601* | 0.01 | 0.04 | 0.13 | (0.01 -1.06) | 0.057 |
| *HLA-C *1602* | 0.01 | 0.01 | 1.07 | (0.11 -10.31) | 0.951 |
| *HLA-C *1701* | 0.02 | 0.01 | 5.78 | (1.43 -23.43) | 0.014 |
| *HLA-DPB1 *0101* | 0.09 | 0.09 | 1.08 | (0.61 -1.91) | 0.801 |
| *HLA-DPB1 *0201* | 0.11 | 0.15 | 0.69 | (0.4 -1.2) | 0.188 |
| *HLA-DPB1 *0202* | 0.01 | 0.01 | 1.56 | (0.24 -10.18) | 0.643 |
| *HLA-DPB1 *0301* | 0.06 | 0.11 | 0.46 | (0.22 -0.96) | 0.039 |
| *HLA-DPB1 *0401* | 0.4 | 0.41 | 0.96 | (0.68 -1.35) | 0.798 |
| *HLA-DPB1 *0402* | 0.09 | 0.11 | 0.8 | (0.45 -1.42) | 0.448 |
| *HLA-DPB1 *0501* | 0.01 | 0.01 | 0.48 | (0.06 -4.02) | 0.496 |
| *HLA-DPB1 *0601* | 0.01 | 0.02 | 0.51 | (0.07 -3.65) | 0.503 |
| *HLA-DPB1 *0901* | 0.02 | 0.01 | 2.13 | (0.51 -8.81) | 0.298 |
| *HLA-DPB1 *1001* | 0.01 | 0.02 | 0.78 | (0.17 -3.62) | 0.750 |
| *HLA-DPB1 *1101* | 0.02 | 0.02 | 1.57 | (0.5 -4.9) | 0.437 |
| *HLA-DPB1 *1301* | 0.01 | 0.01 | 0.4 | (0.05 -3.31) | 0.393 |
| *HLA-DPB1 *1401* | 0.02 | 0.01 | 2.09 | (0.63 -7) | 0.231 |
| *HLA-DPB1 *1501* | 0.01 | 0.01 | 1.17 | (0.12 -11.1) | 0.890 |
| *HLA-DPB1 *1601* | 0.02 | 0.01 | 3.29 | (0.94 -11.45) | 0.061 |
| *HLA-DPB1 *1701* | 0.02 | 0.01 | 1.34 | (0.4 -4.49) | 0.636 |
| *HLA-DPB1 *1901* | 0.01 | 0.01 | 0.45 | (0.05 -3.74) | 0.459 |
| *HLA-DPB1 *2301* | 0.01 | 0.01 | 0.47 | (0.01 -21.68) | 0.700 |
| *HLA-DQB1 *0201* | 0.15 | 0.15 | 1.07 | (0.67 -1.71) | 0.775 |
| *HLA-DQB1 *0202* | 0.03 | 0.08 | 0.35 | (0.14 -0.9) | 0.029 |
| *HLA-DQB1 *0301* | 0.14 | 0.15 | 0.88 | (0.56 -1.41) | 0.604 |
| *HLA-DQB1 *0302* | 0.19 | 0.11 | 1.94 | (1.23 -3.05) | 0.004 |
| *HLA-DQB1 *0303* | 0.01 | 0.05 | 0.26 | (0.06 -1.09) | 0.066 |
| *HLA-DQB1 *0402* | 0.04 | 0.02 | 1.89 | (0.76 -4.68) | 0.168 |
| *HLA-DQB1 *0501* | 0.18 | 0.11 | 1.76 | (1.11 -2.77) | 0.015 |
| *HLA-DQB1 *0502* | 0.01 | 0.02 | 0.33 | (0.04 -2.55) | 0.286 |
| *HLA-DQB1 *0503* | 0.1 | 0.03 | 3.72 | (1.94 -7.14) | 7.97E-05 |

Supplementary table 4. (continued)

| Classical Allele | Frequency | | OR | 95% CI | P-value |
|---|---|---|---|---|---|
| | Cases | Controls | | | |
| *HLA-DQB1 *0602* | 0.06 | 0.13 | 0.37 | (0.18 -0.76) | 0.006 |
| *HLA-DQB1 *0603* | 0.02 | 0.07 | 0.24 | (0.07 -0.81) | 0.021 |
| *HLA-DQB1 *0604* | 0.03 | 0.08 | 0.35 | (0.14 -0.91) | 0.032 |
| *HLA-DRB1 *0101* | 0.04 | 0.04 | 0.99 | (0.36 -2.73) | 0.989 |
| *HLA-DRB1 *0102* | 0.11 | 0.01 | 10.18 | (4.55 -22.76) | 1.58E-08 |
| *HLA-DRB1 *0103* | 0.02 | 0.05 | 0.27 | (0.07 -1.05) | 0.058 |
| *HLA-DRB1 *0301* | 0.15 | 0.14 | 1.1 | (0.69 -1.77) | 0.693 |
| *HLA-DRB1 *0401* | 0.08 | 0.09 | 0.92 | (0.5 -1.71) | 0.798 |
| *HLA-DRB1 *0403* | 0.07 | 0.01 | 29.3 | (8.39 -102.41) | 1.22E-07 |
| *HLA-DRB1 *0404* | 0.03 | 0.03 | 0.88 | (0.32 -2.46) | 0.808 |
| *HLA-DRB1 *0408* | 0.01 | 0.01 | 0.73 | (0.06 -8.3) | 0.801 |
| *HLA-DRB1 *0701* | 0.04 | 0.11 | 0.33 | (0.15 -0.74) | 0.007 |
| *HLA-DRB1 *0801* | 0.02 | 0.02 | 1.4 | (0.47 -4.13) | 0.545 |
| *HLA-DRB1 *0901* | 0.01 | 0.02 | 0.34 | (0.04 -2.7) | 0.310 |
| *HLA-DRB1 *1101* | 0.05 | 0.06 | 0.83 | (0.37 -1.84) | 0.638 |
| *HLA-DRB1 *1104* | 0.04 | 0.02 | 1.82 | (0.71 -4.71) | 0.214 |
| *HLA-DRB1 *1201* | 0.01 | 0.01 | 0.42 | (0.05 -3.72) | 0.437 |
| *HLA-DRB1 *1301* | 0.02 | 0.09 | 0.26 | (0.09 -0.73) | 0.010 |
| *HLA-DRB1 *1302* | 0.04 | 0.07 | 0.54 | (0.23 -1.28) | 0.161 |
| *HLA-DRB1 *1303* | 0.01 | 0.01 | 0.49 | (0.05 -4.67) | 0.535 |
| *HLA-DRB1 *1401* | 0.02 | 0.03 | 0.93 | (0.28 -3.08) | 0.912 |
| *HLA-DRB1 *1501* | 0.04 | 0.14 | 0.24 | (0.1 -0.56) | 0.001 |

**Supplementary table 5**: Sex specific association testing in the HLA region in JIA associated uveitis; idiopathic chronic anterior uveitis and panuveitis.

| **JIA uveitis (n=92)** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Univariable analysis | | | | | | | | | | | |
| | Controls (n=499) | Controls (n=250) | Males (n=27) | | | | Controls (n=249) | Females (n=65) | | | |
| HLA allele | Frequency | Frequency | Frequency | OR | (95% CI) | P | Frequency | Frequency | OR | (95% CI) | P |
| A*02:01 | 0.29 | 0.31 | 0.33 | 1.07 | (0.60-1.92) | 0.25 | 0.2 | 0.45 | 2.30 | (1.51-3.49) | 1.03E-04 |
| DRB1*08:01 | 0.02 | 0.02 | 0.11 | 7.27 | (2.34-22.56) | 5.89E04 | 0.02 | 0.18 | 13.79 | (6.05-31.47) | 4.46E-10 |
| DRB1*11:01 | 0.06 | 0.05 | 0.19 | 6.25 | (2.49-15.72) | 9.71E-05 | 0.07 | 0.05 | 0.62 | (0.24-1.61) | 0.32 |
| DRB1*11:04 | 0.02 | 0.02 | 0.06 | 2.21 | (0.63-7.76) | 0.21 | 0.02 | 0.12 | 10.16 | (3.88-26.58) | 2.32E-06 |
| DQB1*03:01 | 0.15 | 0.14 | 0.35 | 3.33 | (1.76-6.30) | 2.23E-04 | 0.17 | 0.22 | 1.43 | (0.89-2.28) | 0.14 |
| DQB1*04:02 | 0.02 | 0.02 | 0.13 | 7.17 | (2.45-21.00) | 3.25E-04 | 0.02 | 0.17 | 12.23 | (5.41-27.62) | 1.73E-09 |
| **Idiopathic chronic anterior uveitis (no JIA, n=50)** | | | | | | | | | | | |
| Univariable analysis | | | | | | | | | | | |
| | Controls (n=499) | Controls (n=250) | Males (n=17) | | | | Controls (n=249) | Females (n=33) | | | |
| HLA allele | Frequency | Frequency | Frequency | OR | (95% CI) | P | Frequency | Frequency | OR | (95% CI) | P |
| DQB1*05:03 | 0.03 | 0.04 | 0.06 | 1.46 | (0.31-6.87) | 0.63 | 0.01 | 0.12 | 13.00 | (4.17-40.49) | 9.75E-06 |
| DRB1*08:01 | 0.02 | 0.02 | 0.06 | 3.37 | (0.75-15.28) | 0.11 | 0.02 | 0.11 | 6.80 | (2.35-19.68) | 4.12E-04 |
| DQB1*04:02 | 0.02 | 0.02 | 0.09 | 4.11 | (1.13-14.95) | 0.03 | 0.02 | 0.11 | 6.43 | (2.26-18.33) | 4.95E-04 |
| **Panuveitis (n=81)** | | | | | | | | | | | |
| Univariable analysis | | | | | | | | | | | |
| | Controls (n=499) | Controls (n=250) | Males (n=43) | | | | Controls (n=249) | Females (n=38) | | | |
| HLA allele | Frequency | Frequency | Frequency | OR | (95% CI) | P | Frequency | Frequency | OR | (95% CI) | P |
| DRB1*01:02 | 0.01 | 0.01 | 0.08 | 7.25 | (2.19-23.97) | 0.001 | 0.03 | 0.14 | 13.16 | (4.48-38.69) | 2.82E-06 |
| DRB1*04:03 | 0.007 | 0.01 | 0.03 | 15.48 | (1.75-137.31) | 0.01 | 0.01 | 0.12 | 38.27 | (8.25-177.52) | 3.22E-06 |

**Supplementary table 6:** Frequency of polymorphic amino acid positions in the HLA gene and association in cases according to the anatomical location of their inflammation versus healthy controls (n=499).

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**Supplementary table 7A:** HLA amino acid associations before and after conditioning on the primary association in JIA associated uveitis; idiopathic chronic anterior uveitis; intermediate and panuveitis.

| | | | JIA uveitis (n=92) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Univariable analysis | | | Conditional analysis on DRB1*08:01 | | |
| HLA allele | Position | AA | OR | (95% CI) | P | OR | (95% CI) | P |
| *DRB1* | 13 | G | 11.12 | (5.76-21.43) | 6.51E-13 | | NA | NA |
| *DRB1* | 16 | Y | 11.12 | (5.76-21.43) | 6.51E-13 | | NA | NA |
| *DRB1* | 57 | S | 11.12 | (5.76-21.43) | 6.51E-13 | | NA | NA |
| *DRB1* | 67 | F | 11.12 | (5.76-21.43) | 6.51E-13 | | NA | NA |
| *DRB1* | 74 | L | 11.12 | (5.76-21.43) | 6.51E-13 | | NA | NA |
| *DRB1* | 189 | S | 11.12 | (5.76-21.43) | 6.51E-13 | | NA | NA |
| *DQB1* | 9 | F | 10.11 | (5.32-19.22) | 1.69E-12 | 1.51 | (0.09-25.16) | 0.78 |
| *DQB1* | 55 | R | 10.11 | (5.32-19.22) | 1.69E-12 | 1.51 | (0.09-25.16) | 0.78 |
| *DQB1* | 56 | L | 10.11 | (5.32-19.22) | 1.69E-12 | 1.51 | (0.09-25.16) | 0.78 |
| *DQB1* | 70 | E | 10.11 | (5.32-19.22) | 1.69E-12 | 1.51 | (0.09-25.16) | 0.78 |
| *DQB1* | 71 | D | 10.11 | (5.32-19.22) | 1.69E-12 | 1.51 | (0.09-25.16) | 0.78 |
| *DQB1* | 74 | S | 10.11 | (5.32-19.22) | 1.69E-12 | 1.51 | (0.09-25.16) | 0.78 |

| | | | Idiopathic chronic anterior uveitis (No JIA,n=50) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Univariable analysis | | | Conditional analysis on DQB1*04:02 | | |
| HLA allele | Position | AA | OR | (95% CI) | P | OR | (95% CI) | P |
| *DQB1* | 9 | F | 5.37 | (2.38-12.11) | 5.07E-05 | | NA | NA |
| *DQB1* | 55 | R | 5.37 | (2.38-12.11) | 5.07E-05 | | NA | NA |
| *DQB1* | 56 | L | 5.37 | (2.38-12.11) | 5.07E-05 | | NA | NA |
| *DQB1* | 70 | E | 5.37 | (2.38-12.11) | 5.07E-05 | | NA | NA |
| *DQB1* | 71 | D | 5.37 | (2.38-12.11) | 5.07E-05 | | NA | NA |
| *DQB1* | 74 | S | 5.37 | (2.38-12.11) | 5.07E-05 | | NA | NA |
| *DRB1* | 13 | G | 5.30 | (2.26-12.44) | 1.30E-04 | 1.03 | (0.09-12.13) | 0.99 |
| *DRB1* | 16 | Y | 5.30 | (2.26-12.44) | 1.30E-04 | 1.03 | (0.09-12.13) | 0.99 |
| *DRB1* | 57 | S | 5.30 | (2.26-12.44) | 1.30E-04 | 1.03 | (0.09-12.13) | 0.99 |
| *DRB1* | 67 | F | 5.30 | (2.26-12.44) | 1.30E-04 | 1.03 | (0.09-12.13) | 0.99 |
| *DRB1* | 74 | L | 5.30 | (2.26-12.44) | 1.30E-04 | 1.03 | (0.09-12.13) | 0.99 |
| *DRB1* | 189 | S | 5.30 | (2.26-12.44) | 1.30E-04 | 1.03 | (0.09-12.13) | 0.99 |

| | | | Intermediaite uveitis (n=39) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Univariable analysis | | | Conditional analysis on DRB1*15:01 | | |
| HLA allele | Position | AA | OR | (95% CI) | P | OR | (95% CI) | P |
| *DRB1* | 11 | P | 2.18 | (1.26-3.77) | 0.005 | | NA | NA |
| *DRB1* | 13 | R | 2.18 | (1.26-3.77) | 0.005 | | NA | NA |
| *DRB1* | 32 | I | 2.18 | (1.26-3.77) | 0.005 | | NA | NA |
| *DRB1* | 33 | Y | 2.18 | (1.26-3.77) | 0.005 | | NA | NA |
| *DRB1* | 34 | N | 2.18 | (1.26-3.77) | 0.005 | | NA | NA |
| *DRB1* | 71 | A | 2.18 | (1.26-3.77) | 0.005 | | NA | NA |
| *DRB1* | 133 | L | 2.18 | (1.26-3.77) | 0.005 | | NA | NA |
| *DRB1* | 142 | M | 2.18 | (1.26-3.77) | 0.005 | | NA | NA |

| | | | Panuveitis (n=81) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Univariable analysis | | | Conditional analysis on DRB1*01:02 | | | Conditional analysis on DRB1*04:03 | | |
| HLA allele | Position | AA | OR | (95% CI) | P | OR | (95% CI) | P | OR | (95% CI) | ***P*** |
| *DRB1* | 85 | A | 10.18 | (4.55-22.76) | 1.58E-08 | | NA | NA | | NA | NA |
| *DRB1* | 74 | E | 29.30 | (8.39-102.41) | 1.22E-07 | 29.61 | (7.96-110.22) | 4.35E-07 | | NA | NA |

**Supplementary table 7B:** Association for unique amino acid positions in *HLA-DRB1*.

| *DRB1* Allele | 11 | 13 | 13 | 16 | 16 | 32 | 33 | 34 | 57 | 57 | 67 | 67 | 71 | 74 | 74 | 74 | 85 | 133 | 142 | 189 | 189 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *01:01 | L | F | | H | | Y | N | Q | D | | L | | R | A | | | V | R | V | R | |
| *01:02 | L | F | | H | | Y | N | Q | D | | L | | R | A | | | A | R | V | R | |
| *03:01 | S | S | | H | | H | N | Q | D | | L | | K | R | | | V | R | V | R | |
| *04:01 | V | H | | H | | Y | H | Q | D | | L | | K | A | | | V | R | V | R | |
| *04:03 | V | H | | H | | Y | H | Q | D | | L | | R | E | | | V | R | V | R | |
| *07:01 | G | Y | | H | | Y | N | Q | V | | I | | R | Q | | | V | R | V | R | |
| ***08:01** ***08:01** | S | **G** | **G** | **Y** | **Y** | Y | N | Q | **S** | **S** | **F** | **F** | R | **L** | L | | V | R | V | **S** | **S** |
| *09:01 | D | F | | H | | Y | N | Q | V | | **F** | **F** | R | E | | | V | R | V | R | |
| *10:01 | V | F | | H | | H | N | Q | D | | L | | R | A | | | V | R | V | R | |
| *11:01 | S | S | | H | | Y | N | Q | D | | **F** | **F** | R | A | | | V | R | V | R | |
| *11:04 | S | S | | H | | Y | N | Q | D | | **F** | **F** | R | A | | | V | R | V | R | |
| *12:01 | S | **G** | **G** | **Y** | **Y** | H | N | Q | V | | I | | R | A | | | A | R | V | R | |
| *13:01 | S | S | | H | | H | N | Q | D | | I | | E | A | | | V | R | V | R | |
| *14:01 | S | S | | H | | H | N | Q | A | | L | | R | E | | | V | R | V | R | |
| ***15:01** | P | R | | H | | I | Y | N | D | | I | | A | A | | | V | **L** | **M** | R | |
| *16:01 | P | R | | H | | I | Y | N | D | | F | **F** | R | A | | | V | **L** | **M** | R | |

The colors represent associated *HLA* alleles and amino acids for the different subgroups of uveitis.

**JIA-associated uveitis**

Idiopathic chronic anterior uveitis

Intermediate uveitis

Panuveitis

**Supplementary table 8:** Used peptides (n=157) with high affinity for the HLA-DQ*05 alleles based on proteome data from HLA ligand atlas.[35]

| Peptide |
|---|
| RRREKVGVIVDVLSDT |
| GIVRNWDDMKHLWD |
| RRREKVGVIVDVLSDTA |
| RREKVGVIVDVLSDT |
| LNEDLRSWTAADMAAQITKRKWEAA |
| YPGSIEVRWFRNGQEEKAG |
| GRGEPRFISVGYVDDTQFV |
| EEGVRRALDFAVGEYNKA |
| KQIQQYMKIISSFKNKEDQYD |
| ETPVFYKLVPDPV |
| LGLAVGSYLVRRSRRPQ |
| LGELFNPYYDPLQWKSSH |
| LVRPPVQVYGIEGRYA |
| MKPSELLKAEAHHAEL |
| VRPPVQVYGIEGRY |
| YEMPSEEGYQDYEPEA |
| VAKNKDQGTYEDYVEG |
| YLADKSYIEGYVPSQAD |
| NDHFVKLISWYDNEFGYSNRVVDL |
| FRPTLVFRDHHAHL |
| FRPTLVFRDHHAHLF |
| DQFTPVKIEGYEDQVL |
| GPIPEVLKNYMDAQYY |
| FRPTLVFRDHHAH |
| RHPDEAAFFDTASTGKT |
| RHPDEAAFFDTASTGK |
| IQKIISMLQDAEEQQGE |
| PPPYHTFEEPVYMKS |
| GLAVGSYLVRRSRRPQ |
| FHYRQYSAGKAA |
| EVDPNIQAVRTQEKE |
| EKINEGFDLLHSGKS |
| FEKINEGFDLLHSGKS |
| PFEKINEGFDLLHSGKS |
| MKPSELLKAEAHHAE |
| HRHPDEAAFFDTASTGK |
| HPDEAAFFDTASTGKT |
| AMFKVGMFKIHPEIPE |
| LPINSTVEDIAAEE |
| LRDYLHLPPEIVPAT |
| LDYGPFRDVPAY |
| SGYKIYPGHGRR |
| NEDNGIIKAFRNIPGITL |
| TRVALFRDWIDGVLNNPG |
| SSERQDIAVISDSYFPRY |

Supplementary table 8. (continued)

| Peptide |
|---|
| EQHLYYQDQLLPVSRII |
| WKGFTPYYARLGPHTVL |
| GSQYWRFEDGVLDPDYPR |
| STKLKQVEDEKNSF |
| SFDRGTLSGWILSKAKK |
| AFVEFEDPRDAADA |
| RDDGKEALKFYTDPSYF |
| MNSKKYPVAHFIDQTLK |
| KKRTKKFIRHQSDRYVKI |
| DDERRNAEQYKDQADKAST |
| EGPLKGILGYTEHQVV |
| SISSFKDPKIST |
| GDAEEAQIDDEAHPV |
| IKEIEDFDSLEAL |
| TKAVIHWIMDIPF |
| KGGKIGLFGGAGVGK |
| ILREDPAYLHYYDPAGAEDPLGAI |
| KDVIKEIEDFDSLEAL |
| KAKKPKTVKAKPVKA |
| AFVEFEDPRDAED |
| TQKGLKNVFDEAIL |
| IEVFKSSQEEVRSYSDPPLKF |
| LTQKGLKNVFDEAILA |
| AAIPKVKAIYDIERPDL |
| PGVAKSQLLSYIDRLAPRSQY |
| VVFLSPLTTFQDQLNRRGEF |
| GGYDDYNGYNDGYGF |
| NERNINITKDLLDL |
| AGVRGYLDKLEPSKITK |
| DLIATQRDLIVTQR |
| TDEVVRSLIDGILTNKK |
| VYGIKKYGPFVAD |
| PNPALQRFKAFNQRF |
| TDEVVRSLIDGILTNK |
| HPASRSFPDPRGLYH |
| VAGVNYFLDVELGRT |
| ISNNKDQATYEDFVEG |
| ISNNKDQATYEDFVEGL |
| EGPLKGILGYTEHQVVSS |
| GGIFLYPANKKSPNGKLR |
| VRPPVQVYGIEGRYAT |
| EKDMYSFLEDMGLK |
| KTISKAKGQPREPQ |
| RKKNLQYYDISAKSNYNFE |
| HGKVEIIANDQGNRTT |

Supplementary table 8. (continued)

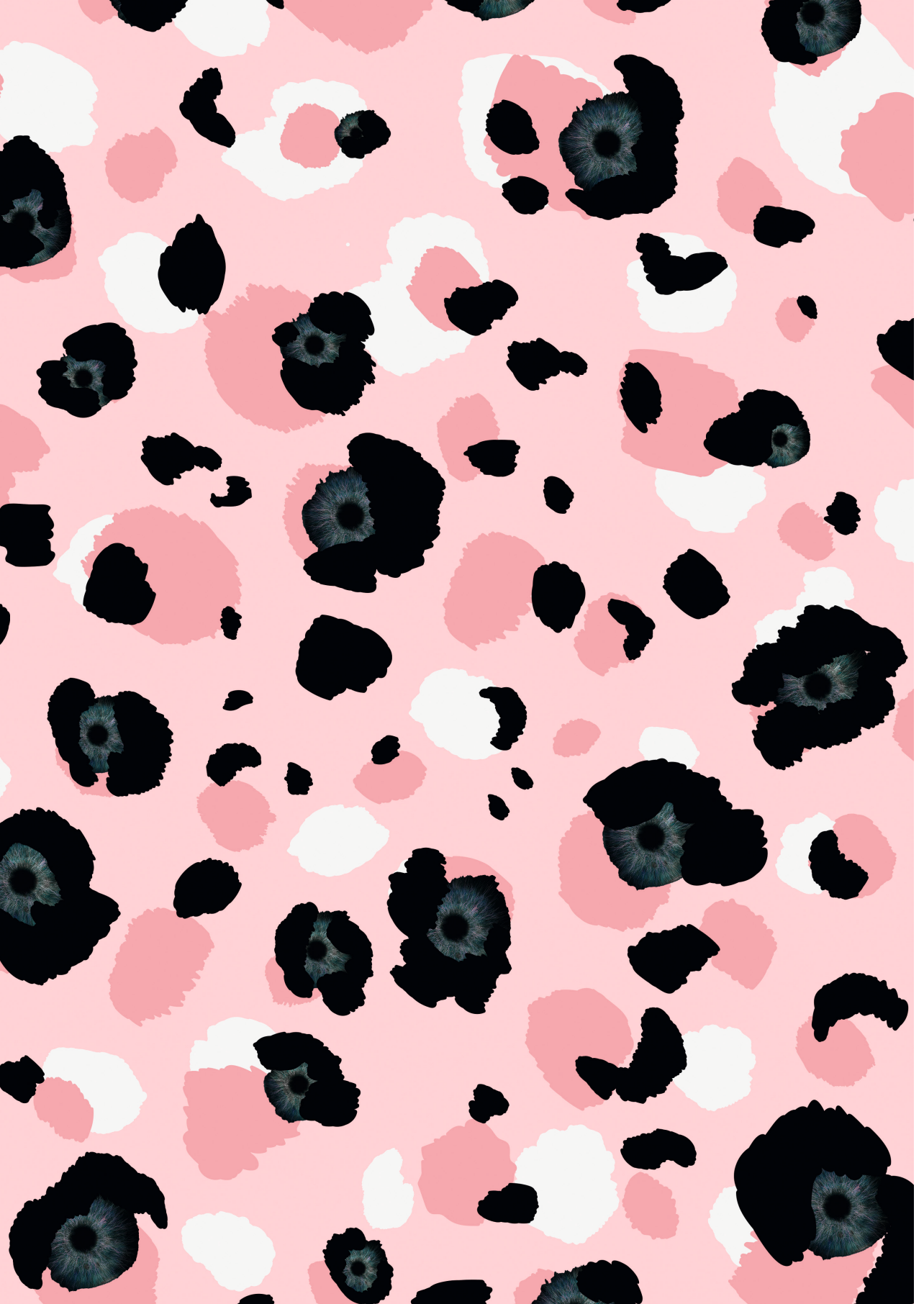
| Peptide |
|---|
| HGKVEIIANDQGNRTTP |
| RQKVKYLQDQLS |
| IRKRNYVRDDAVFIR |
| DVGPDGRLLRGHDQYAYDGKD |
| RSHRIATETDQIGSEII |
| GPDGRLLRGHDQYAYDGKDYI |
| DGRTFYIDHNSKITQW |
| IPKILPWHAGTYS |
| IHSNIYWTDSVLGTV |
| DNIAIIDVPVPSF |
| VGPDGRLLRGHDQYAYDGKDY |
| VPRRVINAINVNHEF |
| VPIEIAPDDPSRKID |
| VPIEIAPDDPSRKIDG |
| GVPIEIAPDDPSRKIDG |
| LSELVQAVSDPSSPQYG |
| IHSNIYWTDSVLGTVS |
| EPPGELADISDVEGEVG |
| KKKLIRDFDEKQQE |
| EKKKLIRDFDEKQQEAN |
| RKLDIKNEDDVKSLSR |
| LAQLARADDYEQVKN |
| SKPRAIVVDPVHGF |
| DVGPDGRLLRGHDQYAYDGKDY |
| SDQIIGRIDDMSSR |
| LDKLIRLVNAQQA |
| NGVPIEIAPDDPSRKIDG |
| DPALDLKVIHLVRDPRAVA |
| KDYIALNEDLRSWTAADTA |
| EPGQRVVVVDDLLATGGT |
| RLKSQDLELSWNLNGLQAD |
| RNDLIVFLADQNAPYFKPK |
| NWNIVSFPVAEELSHH |
| GNLIFDPNNYLPKE |
| DVKDVQMLQDAISKMDPT |
| NWNIVSFPVAEELS |
| AIAKAIDDNMSLDE |
| ALNDHFVKLISWYDNEFGYSNR |
| ALNDHFVKLISWYDNEFGYSNRV |
| ALNDHFVKLISWYDNEFGYSNRVV |
| AAYQKVVAGVANALAHKY |
| ALNDHFVKLISWYDNEFGYSN |
| IEGERELQAFENIEDEIKL |
| IEGERELQAFENIEDE |
| IEGERELQAFENIEDEIK |

Supplementary table 8. (continued)

| Peptide |
|---|
| AVDVRDFSPEDIIVT |
| LLPDIYGWPVATEN |
| GKKVLGAFSDGLAHLDN |
| NDANFDNFVADKDT |
| LVEKSVTGWKEIEYE |
| YIPTNVISITDGQIF |
| QEVLKEMTDGGVD |
| RSPKLFYADHPFIF |
| KSIVDFVKDHGNIK |
| EGDWSWVDDTPFNK |
| GVRNVHTTDFPGNY |
| AMDAPQEVYNDIVSM |
| STDLNRAVDEQGWEY |
| AFVEFEDPRDAAD |
| IALNEDLRSWTAADT |
| YSSPQDLPYPDPAIAQF |
| GDIVWGKVWPDFPD |
| EYMGYIMEKEQAYTDAALNYEMAW |
| FKSKDSEHYKAFEDAAEEFHP |
| FKSKDSEHYKAFEDAAEEFHPYIP |
| TDTDTADQVIASFKVLAGDK |
| KDVTIFSVGVYNANR |

**Supplementary table 9**. Allele frequency of detected *HLA-DRB1* and *DQB1* alleles in European populations. All data are extracted from Hurley et al.[47]

| Allele | Allele count in European populations | Total allele count in European population | Allele frequency | GoNL (imputed) |
|---|---|---|---|---|
| DRB1*01:02 total | 136773 | 11938778 | 0.01 | 0.01 |
| DRB1*04:03 total | 89315 | 11938778 | 0.01 | 0.01 |
| DRB1*08:01 total | 312103 | 11938778 | 0.03 | 0.02 |
| DRB1*11:01 total | 826728 | 11938778 | 0.07 | 0.06 |
| DRB1*11:04 total | 421999 | 11938778 | 0.04 | 0.02 |
| DRB1*15:01 total | 1507494 | 11938778 | 0.13 | 0.14 |
| | | | | |
| DQB1*04:02 total | 341850 | 11735570 | 0.03 | 0.02 |
| DQB1*05:03 total | 310749 | 11735570 | 0.03 | 0.03 |



# CHAPTER 8

## Summary, general discussion and future perspectives

## Fatigue in childhood uveitis

Although uveitis in children is rare, it poses a significant burden on the health-related quality of life (HRQoL) and vision-related quality of life.[1-3] Fatigue is a common distressing symptom in pediatric chronic auto-immune diseases, and has a major impact on the daily life participation of a child.[4] Since fatigue has a major influence on their well-being, it is of great importance to be more aware and to better understand the symptom of fatigue in children with uveitis. To this end, we performed a cross-sectional study of 72 children and adolescents with uveitis (**Chapter 2**). Both parents and children filled out three validated (self-administered) questionnaires: 1) Pediatric Quality of Life Inventory multidimensional fatigue scale (PedsQL-MFS) to measure fatigue, 2) Pediatric Quality of Life Inventory – core generic score (PedsQL-GCS) to measure HRQoL, and 3) visual analogue score (VAS) to measure the pain score. Our study shows that the mean fatigue score and the prevalence of severe fatigue (>2SD below the norm on the total fatigue subscale) in children and adolescents with non-infectious uveitis is comparable to their healthy peers of the same age. Although similar fatigue scores have been reported in a previous Dutch study, this is the first study to investigate fatigue on HRQoL and the effect of disease-related factors on fatigue.[5] Severe fatigue was associated with a lower HRQoL especially on emotional, physical, school and psychosocial functioning. Interestingly, not disease activity or systemic treatment but a higher pain score influenced severe fatigue. These results emphasize the need to routinely assess fatigue and pain with standardized instruments in children with non-infectious uveitis and to develop (preventive) measures to maintain their well-being.

## Improved clinical outcomes in children with JIA-uveitis in the era of new international multidisciplinary guidelines

In **Chapter 3** we aimed to understand the impact of novel treatment regimens and new international multidisciplinary guidelines on the prognosis of children diagnosed with juvenile idiopathic arthritis (JIA) associated uveitis. Not until recently, international consensus guidelines for the diagnosis and management of JIA-uveitis were lacking. In 2012, an international expert panel established the first JIA-uveitis guideline to recommend treatment and management strategies to reduce inflammation and prevent irreversible visual loss.[6] We retrospectively compared a cohort of 61 patients diagnosed before 2010 to a cohort of 82 patients diagnosed in 2010 and after. We

found that patients diagnosed before 2010 were at higher risk for cataract requiring surgery and secondary glaucoma development. In addition, there was a significant reduction in the number of eyes with visual impairment and blindness. This is in line with a previous study that reports a decline in the number of cataracts, secondary glaucoma and visually impaired/blind eyes. Remarkably, the reduction of these complications was most pronounced between 2009 and 2013.[7] This significant decline in ocular complications is suggested to be attributed to more abundant use of immunomodulatory treatment (IMT).[8-11] In addition, the clinical improvement is also influenced by better access to multidisciplinary specialized care between ophthalmologist and pediatric rheumatologists. Although the design of our study cannot be used to identify causality, patients diagnosed after 2010 used IMT more often and IMT was also initiated earlier in the disease course. In addition, they were referred earlier to a tertiary referral center.

In conclusion, our study reports a significant improvement in the prognosis of children with JIA-uveitis in the era of more specialized care. More specifically, we show a significant decrease in the number of cataract surgeries, secondary glaucoma development and lower rates of visual impairment and blindness parallel with more intensive IMT and earlier access to tertiary care.

## New treatment strategies in children with refractory non-anterior uveitis

The registration of tumor necrosis factor alpha (TNF-α) inhibitors has led to a major improvement in the management of children with JIA-uveitis as described in **chapter 3** and previous studies.[11] However, clinical studies in children with uveitis not associated with JIA are scarce.[12] Also, approximately 20% of children with non-anterior uveitis have white matter abnormalities in the brain on MRI which is a relative contraindication for TNF-α inhibitors.[12] In **chapter 4,** we describe seven patients with severe refractory non-anterior uveitis with leakage on fluorescein angiogram (FA) that were off-label treated with tocilizumab (TZC) intravenously every four weeks (eight mg/kg). In all patients there was a significant improvement of macular edema and capillary leakage on FA. Best-corrected visual acuity improved in five eyes and worsened in one eye due to cataract. In conclusion, this small case series showed that treatment with TCZ is successful in children with refractory IU and panuveitis who failed on TNF-α inhibitors or had a relative contra-indication for TNF- α inhibitors.

## Biomarkers as prediction for csDMARD failure in childhood non-infectious uveitis

As outlined in detail in the introduction of this thesis in **chapter 1,** pediatric uveitis is treated by a *step-up* approach consisting of local therapy followed by, if necessary, systemic corticosteroids, switching to systemic immunomodulatory therapy, and finally addition of biological therapy.[6] Approximately in a third of patients the severity of the disease requires them to go through the entire sequence of this approach and require biological treatment to control their ocular inflammation.[13-15] It is currently unknown which patients will be in need of biological therapy. Biomarkers that can identify these patients in advance may aid in early identification and perhaps prompt start of biological therapy in individual cases. With this in mind, we conducted a biomarker study in **chapter 5**. Here**,** we analyzed 368 inflammatory biomarker proteins in serum samples of 72 treatment-free patients with active uveitis (new onset or relapse) and 15 healthy control children. Our results showed that the overall protein profile varied substantially between patients but that this could not be attributed to uveitis subtype. We therefore asked ourselves if this molecular heterogeneity could be linked to the need of biological therapy to control uveitis. To this end, we categorized the 37 patients that were sampled at diagnosis and had sufficiently lengthy follow-up data according to the use of biological therapy after sampling. Since biological therapy is introduced after failure of "conventional synthetic disease modifying antirheumatic drugs (csDMARDS), we labeled these cases as csDMARD "responder" or "csDMARD non-responder". Next, using random forest models, we identified protein signatures at diagnosis that harbored significant discriminative power to stratify in advance patients that did not respond to csDMARDs. More specifically, we were able to design a parsimonious model based on ten serum proteins that could predict csDMARD failure with an overall accuracy of 84%. Patients that were dichotomized by the expression levels of the 10-protein signature at diagnosis showed a large difference in risk for csDMARD failure during follow-up. Thus, this protein signature has a potential predictive capacity for csDMARD failure. Prospective studies are required to further evaluate the robustness and accuracy of the proposed serum protein signature in identification of csDMARD failure and biological therapy need in pediatric uveitis, but serves as a proof of concept study for molecular profiling as a basis for clinical decision tools in pediatric uveitis.

## Heterogeneity in B cell memory genes in patients with JIA-uveitis

Up to one third of children with oligo JIA develop uveitis within four years after arthritis on set.[16-19] Although guidelines aid in the identification of high-risk patients, there is still an unmet need for more accurate prediction tools to refine the current uveitis screening strategies. B cells have been implicated in the pathogenesis of JIA-uveitis.[20] In **Chapter 6**, we performed transcriptome profiling of peripheral blood CD19-positive B cells in patients with JIA with (n=14) and without uveitis (n=13). Head-to-head group comparison of patients with and without uveitis showed no evidence for significant changes in gene expression. However, we observed considerably variation in gene expression levels between uveitis cases. Because we hypothesized that this may be driven by changes in B-cell subsets we aimed to deconvolute the peripheral B cell compartment using transcriptomics data. Indeed, estimation of the leukocyte composition using the bulk transcriptome showed altered proportion of major B cell subsets across the samples. Single cell sequencing studies support that the peripheral blood B cell compartment contains approximately 10 functionally distinct B cell subsets.[21,22] Therefore, we hypothesized that comparison of cases by hallmark genes of B cell subsets may reveal changes in overall B cell composition among patients. Indeed, principal component analysis based on relative expression of 17 key genes of B cell subsets revealed two distinct clusters of JIA-uveitis patients. These two clusters were mostly characterized by contrasting expression of memory B cell genes. More specifically, head-to-head differential gene expression analysis of the CD19+ B cell transcriptomes of these newly PCA-identified JIA-uveitis cluster showed significant changes in functional B cell genes (e.g., *BAIAP3*), which we attributed to increased proportion of memory B cells based on B cell subset deconvolution analysis. In conclusion, heterogeneity in B cell memory genes among patients with uveitis supports a role for memory B cells in the etiology of JIA-uveitis and shows previously underappreciated molecular heterogeneity of uveitis in JIA. The clinical implications of the uveitis clusters require more investigation.

## Shared risk *HLA* alleles between clinically distinct forms of childhood non-infectious uveitis

Pediatric non-infectious uveitis currently consists out of more than five different clinical phenotypes, however the molecular mechanisms that distinguish these

phenotypes are unknown. Several *HLA* risk alleles have been identified for specific types of uveitis, but investigation of genetic linkages with HLA loci across pediatric uveitis subtypes is lacking.[23-27] In **chapter 7**, we performed next-generation full-length sequencing of *HLA-A, HLA-B, HLA-C*, *HLA-DPB1, HLA-DQB1,* and *HLA-DRB1* in 280 patients with pediatric non-infectious uveitis and compared these results with 499 population controls. We confirmed the strong *HLA-DRB1*08:01* and *HLA-DQB1*04:02* association with JIA-uveitis. Remarkably, for idiopathic chronic anterior uveitis (CAU) without arthritis, which is ophthalmological similar to JIA-uveitis, we observed an association for the JIA risk loci *HLA-DQB1*04:02* and *HLA-DRB1*08:01.* The *HLA-DQB1*05:03* allele was also an independent risk allele for CAU as well as a risk allele for panuveitis. This risk allele distinguishes itself from the non-risk alleles (*HLA-DQB1*05:01* and *HLA-DQB1*05:02*) by a allele-defining amino acid located at the edge of the peptide binding groove. Computational modelling of substituting this residue with other amino acids revealed a significant change in peptide binding capacity for *HLA-DQB1*05:03* compared to the non-risk alleles. In conclusion, these results show shared *HLA* associations among clinically distinct phenotypes of pediatric uveitis and highlight genetic predisposition affecting the antigen presentation pathway.

## General discussion and future perspectives

The high variability in clinical presentation and treatment between uveitis subtypes, and between patients, make it challenging to find the optimal management for an individual patient. The goal of treatment is to suppress ocular inflammation with no structural damage to the eye. The development of new IMT agents both dampen the inflammation and prevent long-term treatment with (topical) corticosteroids. This thesis provided new insights in both clinical outcomes as well as molecular pathways and is a stepping stone towards a tool for clinical decision making at the individual level in children with non-infectious. However, future research is needed to identify a biological profile that guide us to the realization of personalized medicine.

## A personalized medicine approach in childhood non-infectious uveitis

The term *personalized medicine* refers to an approach for disease prevention and tailor-made treatment for individual patients. Often, this takes into account the biological fingerprint of patients established by mathematical and statistical inference

of multi-omics that captures an individual's genetic make-up, variability in gene, protein, and metabolite expression, and environmental exposure (**Figure 1**).

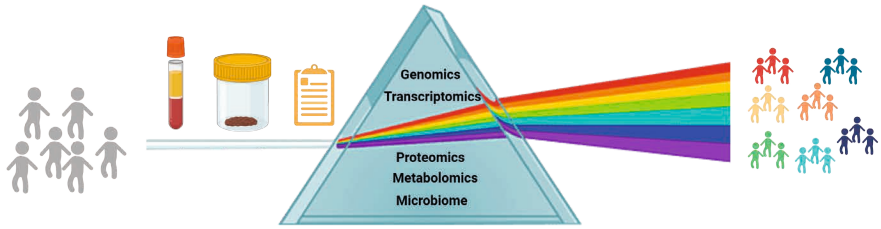



**Figure 1.** A personalized medicine approach in children with non-infectious uveitis.
Using high-throughput techniques analyzing layers of the immune system yields a biological profile of individuals that can be exploited for the development of novel biomarkers. Illustration created with BioRender.com

Whatever the technological approach, the goal of personalized medicine is to prevent, diagnose, treat and cure at an individual's level. Over the last decades, the application of a variety of *omics* techniques (e.g., transcriptomics, proteomics, metabolomics), identified an ensemble of molecular markers associated with uveitis in children, but these studies are mostly limited to patients with JIA.[28] In this thesis, we extended the molecular profiling of pediatric uveitis and provided a proof of concept for molecular-based clinical decision tools that aid in the realization of personalized medicine for the care of childhood uveitis. Overall, our studies demonstrated the need for individual based risk assessment tools and potential reclassification of cases based on disease mechanisms rather than over the current clinical classification systems. This is illustrated by the fact that we detected shared *HLA* associations among clinically distinct phenotypes of pediatric uveitis and highlight genetic predisposition affecting the antigen presentation pathway. We also identified heterogeneity in B cell memory genes among patients with JIA-uveitis, underscoring that there may also be molecular subtypes of uveitis in these cases. Although our study lacked sufficient power to integrate these data layers with clinical data for detailed subgroup analysis (other than several treatment groups), it emphasizes the need for a patient-centric approach rather than population-based inference. We showed that children with uveitis frequently require systemic corticosteroids and/or IMT to preserve vision, which reflects the severity of the inflammation. However, we also showed that treatment response varies widely among children with uveitis. In **Chapter 5**, we identified that biomarkers (regardless of uveitis subtype) can stratify patients at risk for csDMARD failure. In the light of these results, we propose

that more studies use high-throughput techniques to analyze several layers of the immune system (i.e. transcriptome, proteome, metabolome) in combination with computational modeling to unravel disease pathways and to classify patients for a more tailor-made care and personalized care.

The nature of non-infectious uveitis is complex and therefore understanding only one aspect of the pathogenesis will not significantly improve clinical care. Therefore, it is crucial to analyze the interaction between the different biological layers in order to understand the etiology. A systems immunology approach aiming to integrate multi-omics and clinical data can be used to resolve the complexity of the disease etiology and its heterogeneous outcomes. But this approach may also discover novel biomarkers that can be used to guide personalized care. For example, multi-omics analysis of peripheral blood samples of patients with rheumatoid arthritis (RA) was able to predict drug response. That is, drugs treatment altered the molecular profile closer to healthy controls. This alteration was associated with a long-term stabile disease.[29]

In another study, transcriptomic and epigenetic profiling of immune cell types were able to predict clinical response to biological therapy in RA patients.[30] Lastly, a biological profile based on proteomic, metabolomic, and microbial biomarkers was associated with a pro-inflammatory state in quiescent inflammatory bowel disease and predisposes a clinical relapse.[31]

## Potential new targets for personalized medicine in childhood non-infectious uveitis

### Profiling infection history in childhood non-infectious uveitis

Although several studies suggest that both the innate and adaptive immune response play a role in the pathogenesis, the trigger that causes a derailed inflammation is unknown.[20,32-34] Therefore, next to omics technologies to unravel the derailed immunity and to identify disease specific networks/pathways, it is also important to analyze environmental factors that might trigger this derailed immunity. It is hypothesized that an exaggerated or dysregulated host response to an infection may causes a chronic inflammation and tissue damage. A reactivation of persistent microbial agents or inadequately cleared antigen, may intermittently disrupt the regulatory and effector T-cell balance.[35] Other studies further show the role of exposure to

commensal or infectious agents in the etiology of pediatric uveitis which may have been underestimated so far. The identification of high and specific anti-parvovirus B19 antibodies in aqueous humor in patients with JIA-uveitis supports this hypothesis.[36] In addition, previous work from our group demonstrated that a fraction of children with idiopathic uveitis have high intraocular IgG against Toxocara canis, suggesting that exposure to a subclinical infection may provoke chronic inflammatory eye conditions.[37] With the recent advent of novel multiplexed serological techniques, the history of pathogen infection in childhood uveitis can be determined. Identification of a chronic subclinical or relapsing viral infections that maintain an auto-immune response, antiviral treatment or vaccination if available, might positively influence the auto-immune response.

**Profiling commensal microbiota in childhood non-infectious uveitis**

The gut microbiota refers to all different kinds of micro-organisms colonizing in the intestinal tract and has many physiological functions including regulation of the immune system. The intestinal microbiome has been proposed to play a crucial role in the development of non-infectious uveitis in adults.[38] The exact underlying mechanisms are not completely understood but antigenic mimicry, disturbance of intestinal immune homeostasis, destruction of the intestinal barrier and reduction in beneficial anti-inflammatory metabolites are hypothesized. [38] Knowledge about the role of the intestinal microbiome in pediatric uveitis is essential to identify harmful changes in the composition that can trigger immune activation. Especially since targeted interventions such as, oral antibiotics and fecal microbial transplantation to restore the microbiome, have proven to be effective in uveitis models.[38]

## A multidisciplinary approach to the realization of personalized medicine



Although potential biomarkers that better predict uveitis in JIA have been identified (e.g., erythrocyte sedimentation rate and S100A12), these have not yet been implemented in clinical guidelines which highlights one of the major challenges of biomarker research.[28] There are multiple challenges associated with different phases of the biomarker discovery pipeline i.e. pre-analytical (e.g. sample collection), biological diversities (sociodemographic background), disease heterogeneity, and technical limitations.[39] Also, small cohorts of patients make it difficult to replicate and validate potential biomarkers. [39] To overcame these challenges, there is an unmet

need for an international interdisciplinary approach. Different international initiatives of ophthalmologists and pediatric rheumatologists originated in the *Multidisciplinary Working group for Uveitis in Childhood (*MIWGUC) to improve (treatment) guidelines in children with uveitis. However, these collaborations are also fundamental in the improvement of research in this relatively rare disease. Multidisciplinary international teams facilitate sufficiently powered patient cohorts with large amounts of patient material, which is often difficult to obtain due to ethical issues, enables similar standard operating procedures, and easier access to financial resources required to conduct well-powered studies. The feasibility of this collaboration has been demonstrated by a large genetic cohort study in JIA patients and by the development of an international vision-related quality of life questionnaire.[40] Our ambition is to improve research in children with uveitis by promoting these extensive international multicenter collaborations.

## Shared clinical decision-making

Besides the biological profile, it is also important to incorporate patient's preferences (i.e. patient reported outcomes) to identify the optimal care for each individual. Treatment with systemic corticosteroids and/or IMT and other disease-related factors seem to have little impact on the well-being regarding health-related quality of life and fatigue of the child. However, these questionnaires might underestimate the effect of systemic treatment on the child and don't entirely engage the child's perspective. Semi-structured, in-depth interviews with children with a chronic disease have shown that their perspective on full participation in the daily life is having a sense of belonging, the ability to affect social interactions, and the capacity to keep up with peers rather than engaging in activities.[41] This type of measuring patient reported outcomes (i.e. interviews) should be used for future research to gain a wider view of the effect the disease on the child's daily life. This requires an active collaboration between children, healthcare providers, parents, and caregivers. Understanding the child's perspective will give a unique insight in what they value and may help to tailor care to the individual child's need and might also improve treatment compliance.

## Implementations of personalized medicine

A more personalized approach might change the contemporary treatment recommendations from *step-up* to a *step-down* approach. In other words, patients

that are identified as being at risk might benefit from more intensive IMT early in the disease course. Initiation of IMT at an early stage of the disease might allow modulation of biological processes while the disease is in a less mature and more reversible stage. This phase is also referred as the *window of opportunity.* It is therefore tempting to speculate that a rapid control of the severe inflammation with highly effective treatment might be associated with a higher success rate in the attempt to discontinue these heavy medications. Studies in patients with rheumatoid arthritis show that a prolonged symptom duration is associated with a lower change on csDMARD-free sustained remission.[42] Evaluation of this window of opportunity, and the time frame, is left to be explored in future studies.

To summarize, this thesis shows that the multidisciplinary approach and the introduction of new beneficial agents has led to improved clinical outcomes. However, the high variability in presentation and treatment response make it challenging to find the optimal treatment at the individual level. To this end, we provided a framework towards a more personalized care in children with uveitis with special attention for impact on daily live. Future studies using standardized multi-omics and profiling the infection history and microbiome in an internal multidisciplinary setting to characterize patients and identity biomarkers will guide us to the realization of personalized medicine for children with non-infectious uveitis.

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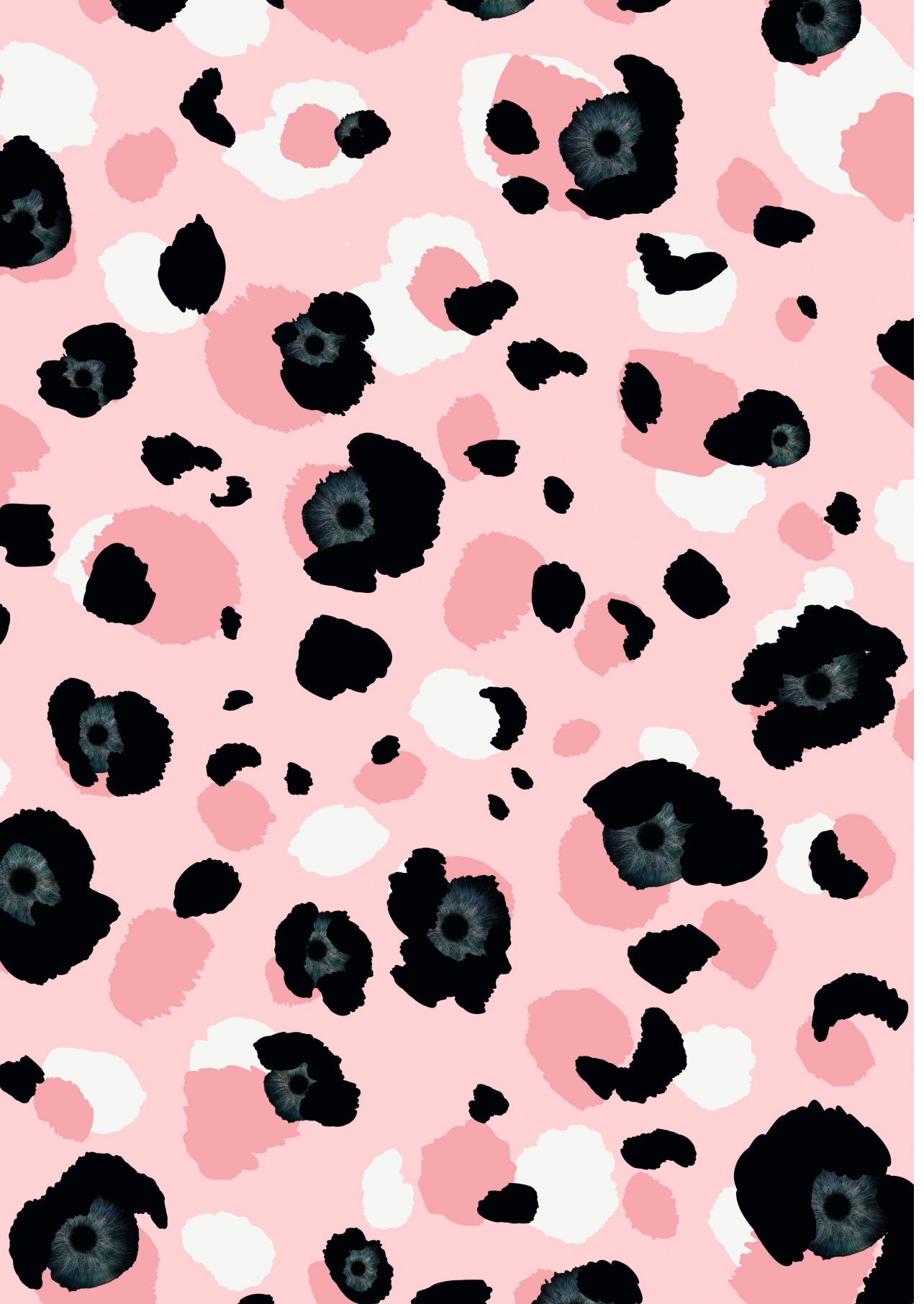


# CHAPTER 9

## Nederlandse samenvatting

# Introductie

In **hoofdstuk 1** van dit proefschrift wordt een algemene introductie gegeven over uveïtis bij kinderen. Uveïtis is een ernstige inwendige oogaandiening die wordt gekenmerkt door een ontsteking van de iris, het vaatvlies en/of het netvlies. Uveïtis op kinderleeftijd is zeldzaam, maar kan leiden tot ernstige complicaties zoals cataract en glaucoom met als gevolg ernstig visusverlies of zelfs blindheid. Bij een merendeel van de patiënten vindt men geen infectieuze oorzaak en wordt daarom geclassificeerd als "niet-infectieus". Niet-infectieuze uveïtis kan zich geïsoleerd presenteren maar kan ook geassocieerd zijn met een systemische aandoening waarvan juveniele idiopathische artritis (JIA) de meest voorkomende oorzaak is. De exacte pathogenese van een niet-infectieuze oogontsteking bij kinderen is nog niet bekend, maar verschillende factoren van het immuunsysteem (bijvoorbeeld witte bloedcellen), maar ook invloeden van buitenaf, lijken een rol te spelen. Het doel van de behandeling is daarom ook om de oogontsteking te onderdrukken en schade aan het oog te voorkomen. Lokale corticosteroïde oogdruppels zijn vaak de eerstelijnsbehandeling (zie **figuur 3** in **hoofdstuk 1**). Echter, systemische behandeling met immunosuppressiva, zoals methotrexaat, is in merendeel van de patiënten noodzakelijk en worden tegenwoordig eerder in het ziekteverloop opgestart. De recente ontwikkeling van nieuwe medicijnen, zoals tumor necrose factor alfa (TNF-α) remmers en interleukine-6 remmers, hebben een belangrijke rol gespeeld in de verbetering van de behandelstrategie en prognose. De grote variabiliteit in klinische presentatie, behandeling tussen verschillende vormen van uveïtis, en tussen patiënten onderling, maken het een uitdaging om de optimale behandeling per individuele patiënt te vinden.

Het doel van dit proefschrift is om in het licht van de ontwikkeling van nieuwe behandelingen en richtlijnen een nieuw en beter beeld te krijgen van de ziektelast, het klinische beloop en prognose van kinderen met uveïtis. Aanvullend hebben we aan de hand van nieuwe technieken het ziektemechanisme verder ontrafeld zodat patiënten in de toekomst beter op maat kunnen worden behandeld.

## Vermoeidheid bij kinderen met niet-infectieuze uveïtis

In **hoofdstuk 2** hebben we gekeken naar de ernst en de prevalentie van vermoeidheid bij kinderen met uveïtis. Vermoeidheid heeft namelijk een grote

impact op de dagelijkse participatie van een kind. In dit cross-sectioneel onderzoek hebben 72 kinderen en adolescenten drie gevalideerde vragenlijsten ingevuld: 1) Pediatric Quality of Life Inventory multidimensional fatigue scale (PedsQL-MFS) om vermoeidheid te meten, 2) Pediatric Quality of Life Inventory - core generic score (PedsQL-GCS) om de kwaliteit van leven te meten, en 3) visual analogue score (VAS) om de pijnscore te meten. Onze studie toont aan dat de gemiddelde vermoeidheidsscore en de prevalentie van ernstige vermoeidheid (>2SD onder de norm op de schaal algemene vermoeidheid) bij kinderen en adolescenten met niet-infectieuze uveïtis vergelijkbaar is met gezonde leeftijdsgenoten (3% vs 4% en 10% vs 7%). Ernstige vermoeidheid was geassocieerd met een lagere kwaliteit van leven, met name op emotioneel, fysiek, school en psychosociaal functioneren. Interessant is dat een hogere pijnscore (odds ratio 1.4; 95% CI 1.06-1.93) samen hangt met ernstige vermoeidheid in plaats van ziekteactiviteit of systemische behandeling. Deze resultaten benadrukken de noodzaak om routinematig vermoeidheid en pijn te beoordelen met gestandaardiseerde instrumenten bij kinderen met niet-infectieuze uveïtis en om (preventieve) maatregelen te ontwikkelen om hun welzijn te waarborgen.

## Klinisch beloop en prognose van kinderen met JIA-uveïtis in het tijdperk van nieuwe internationale en multidisciplinaire richtlijnen

In **hoofdstuk 3** was ons doel om inzicht te krijgen in het effect van nieuwe behandelingsstrategieën en nieuwe internationale en multidisciplinaire richtlijnen op de prognose van kinderen met JIA-uveïtis. In dit retrospectieve onderzoek hebben we een cohort van 61 patiënten die gediagnosticeerd zijn met uveïtis vóór 2010 vergeleken met een cohort van 82 patiënten die gediagnosticeerd zijn in 2010 en daarna. Wij vonden dat patiënten die vóór 2010 waren gediagnosticeerd een hoger risico hadden op cataractchirurgie (hazard ratio 1.84; 95% CI 1.06-3.20) en op de ontwikkeling van secundair glaucoom (hazard ratio 2.81; 95% CI 1.36-5.79). Bovendien was er een significante afname van het aantal ogen met ernstig visusverlies (7% vs 2%) en blindheid (8% vs 0%). Deze significante afname van oculaire complicaties kan mogelijk worden verklaard door het vaker en eerder starten van immunosuppressiva. Daarnaast wordt de klinische verbetering mogelijk ook beïnvloed door een betere toegang tot multidisciplinaire gespecialiseerde zorg tussen oogarts en kinderreumatologen. Hoewel de opzet van onze studie niet kan worden gebruikt

om causaliteit vast te stellen, gebruikten patiënten gediagnosticeerd na 2010 vaker immunosuppressiva (57% vs 98%) en werd de immunosuppressiva ook eerder in het ziektebeloop gestart. Bovendien werden zij eerder doorverwezen naar een tertiair verwijzingscentrum.

Concluderend, onze studie toont een significante verbetering in de prognose van kinderen met JIA-uveïtis in het tijdperk van meer gespecialiseerde zorg.

## Nieuwe behandelingsstrategie voor kinderen met ernstige therapieresistente niet-anterieure uveïtis

De registratie van TNF-α remmers heeft geleid tot een belangrijke verbetering in de behandeling van uveïtis. Echter, ongeveer 20% van de kinderen met niet-anterieure uveïtis hebben witte stof afwijkingen in de hersenen op MRI, wat een relatieve contra-indicatie is voor TNF-α remmers. In **hoofdstuk 4** beschrijven wij zeven patiënten met ernstige refractaire niet-anterieure uveïtis met lekkage op fluoresceïne angiogram (FA) die off-label werden behandeld met intraveneus tocilizumab (TZC) (om de vier weken [acht mg/kg]). Bij alle patiënten was er een significante afname van macula oedeem en capillaire lekkage op FA. De best-gecorrigeerde visus verbeterde in vijf ogen en verslechterde in één oog als gevolg van cataract. Concluderend, deze kleine case serie toont aan dat behandeling met TCZ succesvol is bij kinderen met refractaire niet-anterieure uveïtis die faalden op TNF-α remmers of een relatieve contra-indicatie hadden voor TNF- α remmers.

## Biomarkers voor de voorspelling van csDMARD falen bij kinderen met niet-infectieuze uveïtis

In **hoofdstuk 5** was ons doel om biomarkers te identificeren die kunnen voorspellen welke kinderen uiteindelijk onvoldoende reageren op conventional synthetic disease modifying antirheumatic drugs (csDMARD) en aanvullende behandeling nodig hebben met biologics (bijv. TNF-α remmers). In deze studie hebben we 368 inflammatoire eiwitten in serum geanalyseerd van 72 patiënten met een actieve uveïtis (bij diagnose of recidief) en 15 gezonde kinderen. Onze resultaten toonden aan dat het algemene eiwitprofiel aanzienlijk varieerde tussen patiënten, maar dit kon niet worden toegeschreven aan de verschillende van vormen van uveïtis. Wij vroegen ons daarom af of deze moleculaire heterogeniteit in verband kon worden

gebracht met behandelrespons op csDMARDs. Om dit te kunnen analyseren hebben we de 37 patiënten die gesampled zijn bij diagnose gecategoriseerd in "csDMARD responder" of "csDMARD non-responder". Met behulp van random forest modellen, hebben we een eiwitprofiel van tien eiwitten geïdentificeerd dat csDMARD falen kon voorspellen met een totale nauwkeurigheid van 84%. Interessant is dat patiënten die gesplitst werden op basis van de expressie van de tien eiwitten toonden een groot verschil in risico op csDMARD falen tijdens follow-up. Concluderend, dit eiwitprofiel heeft potentieel een voorspellend vermogen voor csDMARD falen. Dit is een stap richting de ontwikkeling van een meer patiëntgerichte zorg.

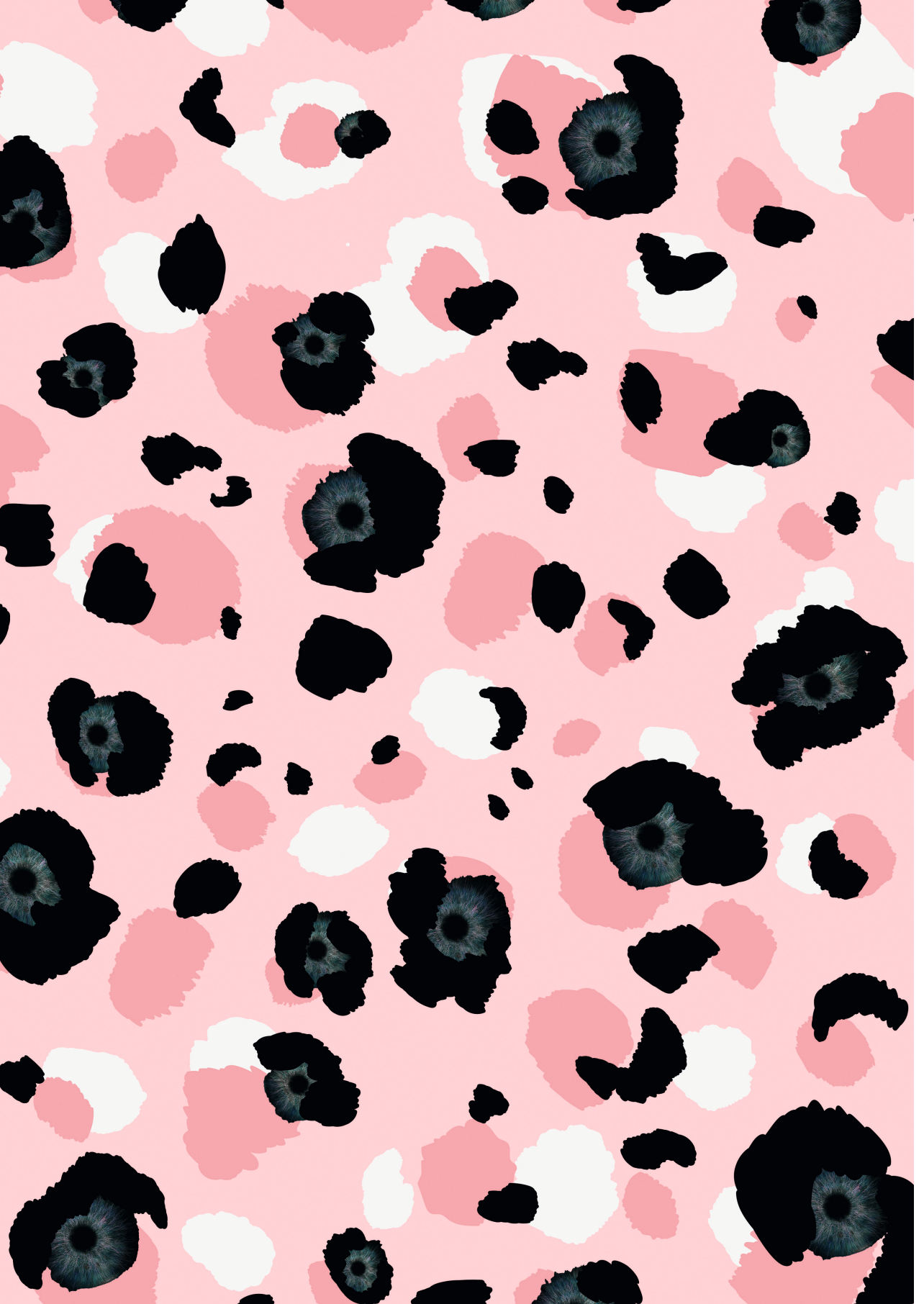
## Heterogeniteit in B-geheugencel genen bij patiënten met JIA-uveïtis

B-cellen spelen mogelijk een rol bij de pathogenese van JIA-uveïtis. Om het ziekteproces van JIA-uveïtis beter te begrijpen hebben we in **hoofdstuk 6** de genexpressie van CD19-positieve B cellen onderzocht bij patiënten met JIA met (n=14) en zonder uveïtis (n=13). Er werden geen significante verschillen gezien in genexpressie tussen de twee groepen. Wij zagen echter een aanzienlijke variatie in genexpressie tussen de uveïtis patiënten die mogelijk verklaard werd door veranderingen in B-cel subsets. Single cell sequencing studies ondersteunen dat er ongeveer 10 verschillende B-cel subsets zijn. Daarom veronderstelden wij dat vergelijking van patiënten op basis van kenmerkende genen van B-cel subsets veranderingen in de algemene B-cel samenstelling tussen patiënten kan onthullen. Interessant, principal component analysis op basis van de genexpressie van deze kenmerkende genen van B-cellen onthulde twee verschillende clusters van patiënten met JIA-uveïtis. Deze twee clusters werden vooral gekarakteriseerd door een verschillende expressie in B-geheugencel genen. Concluderend, deze studie toont heterogeniteit in B-cellen bij patiënten met JIA-uveïtis. Deze data suggereert een rol voor specifieke B-cel subsets in de etiologie van uveïtis in JIA.

## HLA associaties bij verschillende vormen van niet-infectieuze uveïtis bij kinderen

Uveïtis bij kinderen kan zich in verschillende klinische vormen uiten, maar de onderliggende moleculaire mechanismen die deze fenotypes onderscheiden zijn nog onbekend. Verschillende *HLA* allelen zijn geassocieerd met verschillende

vormen van uveïtis. In **hoofdstuk 7** hebben wij bij 280 kinderen met verschillende vormen van uveïtis en 499 gezonde controles alle klassieke *HLA*-allelen geanalyseerd door middel van next-generation full-length sequencing. Onze data bevestigden de eerder gerapporteerde associatie van *HLA-DRB1*08:01* en *HLA-DQB1*04:02* met JIA-uveïtis. Opmerkelijk is dat we voor idiopathische chronische anterieure uveïtis (CAU) zonder artritis, die klinisch identiek is aan JIA-uveïtis, een associatie zagen voor de JIA risico allelen *HLA-DQB1*04:02* en *HLA-DRB1*08:01*. Het *HLA-DQB1*05:03* allel was ook een onafhankelijk risico allel voor CAU, evenals een risico allel voor panuveïtis. Dit risico allel onderscheidt zich van de niet-risico-allelen (*HLA-DQB1*05:01* en *HLA-DQB1*05:02)* door het hebben van een ander aminozuur dat zich bevindt op het eiwit-bindende deel. Deze verandering zorgt voor een significante verandering in de eiwitbinding. Concluderend, deze resultaten laten zien dat klinisch verschillende fenotypes dezelfde *HLA* associaties hebben en benadrukken dat deze genetische predisposities de antigeenpresentatie beïnvloeden.



# APPENDICES

## Dankwoord

Het schrijven van een proefschrift is een team effort. Direct en indirect hebben vele mensen mij de afgelopen vier jaar geholpen. Ik wil daarom graag iedereen bedanken die heeft bijgedragen aan de totstandkoming ervan. Daarbij wil ik graag tot een aantal mensen een persoonlijk dankwoord richten.

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## ABOUT THE AUTHOR

Roos Wennink was born on 11th of December 1989 in Oss, the Netherlands. In 2006 she received the International Baccalaureate Certificate and in 2008 she graduated from secondary school at the Maasland College in Oss. That same year she started Medical School at the University of Utrecht.

Throughout her years in Medical School she explored her interest in research at the department of Surgical Oncology at the UMC Utrecht which resulted in two publications and a book chapter. In 2015 she received her medical degree and started to work as surgical resident (not in training) in the UMC Utrecht. After two years of clinical experience she started her PhD project at the Ophthalmology department and at the Center for Translational Immunology (CTI) under supervision of prof. dr. J.H. de Boer, dr. J.J.W. Kuiper and dr. V. Kalinina Ayuso. During her PhD she received the Bayer Ophthalmology Researsch Award (BORA) of €25.000 with the aim to identify biological mediators that may assist in the prediction of therapy response. This resulted in a publication in Translation Vision Science & Technology (**Chapter 5**). She also received an international Association for Research in Vision and Ophthalmology (ARVO) travel grant to speak at the ARVO conference in 2021. Alongside her research, she was the chairman of the Dutch Ophthalmology PhD Students (DOPS) conference in 2020. In March 2022, she will take the next step and she will start her residency in ophthalmology at the UMC Utrecht.

# LIST OF PUBLICATIONS

**This thesis**

**Wennink RAW**, Pandit A, Haasnoot AJW, Hiddingh S, Kalinina Ayuso V, Wulffraat NM, Vastert BJ, Radstake TRDJ, de Boer JH, Kuiper JJW. Whole Transcriptome Analysis Reveals Heterogeneity in B Cell Memory Populations in Patients With Juvenile Idiopathic Arthritis-Associated Uveitis. Front Immunol. 2020 Sep 17;11:2170. doi: 10.3389/fimmu.2020.02170. PMID: 33042130; PMCID: PMC7527539.

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