Genetics in juvenile idiopathic arthritis

Albers, H.M.

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

In this thesis the uncommon childhood disease Juvenile Idiopathic Arthritis (JIA) was studied. In a large cohort of JIA patients the association of genetic markers with the susceptibility to JIA was investigated. Associated markers would represent the genetic predisposition for developing disease, but could also be helpful in elucidating the pathophysiology of JIA, which is still largely unknown. Additionally, genetic markers have been studied in relation to the course of disease in order to identify prognostic markers for disease severity. Finally the role of genetic parameters in the ability to predict the response to treatment was examined with the aim of serving the pediatric rheumatologist in selecting the best individual treatment.

Definition and classification of JIA

About 100 years ago the first attempt was made to classify chronic arthritis in children, although the first report of juvenile arthritis by Boticelli dates back to 1483.[1] From the 1940's in Europe a classification of Juvenile Chronic Arthritis (JCA) was used, as defined by the European League against Rheumatism (EULAR).[2] In North America the nomenclature of the American Rheumatism Association (ACR), defining Juvenile Rheumatoid Arthritis (JRA), has been adhered to.[3] In the following years more distinct phenotypes were recognized, for example juvenile onset of spondyloarthritis, arthritis associated with psoriasis and progression of oligoarthritis into a polyarticular course. In 1994 the International League of Associations for Rheumatology (ILAR) was founded and created a worldwide consensus on re-classifying juvenile arthritis as Juvenile Idiopathic Arthritis (JIA). The first version of this JIA classification was proposed in 1994[4] which was subsequently revised, incorporating the results of several studies validating these classification criteria.[5;6]

JIA is defined as arthritis of unknown etiology that begins before the sixteenth birthday and persists for at least 6 weeks with other known conditions having been excluded. JIA encompasses a group of heterogeneous diseases that are characterized by chronic arthritis. The ILAR classification is based predominantly on clinical features and some laboratory parameters and has the intention to define homogeneous subtypes (Table 1). The major criterion is the number of affected joints present at disease onset.

Systemic JIA accounts for 10-15% of patients with JIA. Nowadays systemic JIA is considered to be an auto-inflammatory disease or syndrome due to the activation of the innate immune system and the prominent role of IL-1 and IL-6.[7] The course of disease can be monocyclic, can follow a relapsing course or be continuously active. The subtypes oligoarthritis (persistent and extended) and rheumatoid factor (RF) negative polyarthritis share many clinical features. Antinuclear antibodies (ANA) are predominantly present in these subtypes. Oligoarthritis has a higher prevalence in the Western world and comprises up to 50% of the JIA patients. JIA-associated uveitis is an extra-articular manifestation, which mainly develops in patients with oligoarthritis (10-30%) and will be discussed in more detail later on. Almost 20% of the total group of JIA patients has RF-negative polyarthritis. RF-positive polyarthritis is the juvenile equivalent of adult rheumatoid arthritis (RA) and is present in only a small percentage (about 5%) of JIA patients. It is the only subtype in which antibodies to cyclic citrullinated peptides (anti-CCP) are found.[8] Psoriatic arthritis (5-10% of all JIA patients) represents a heterogeneous group of patients. Two categories seem to be present: patients with psoriasis and arthritis resembling enthesitis related arthritis and patients that have early-onset, ANA positive oligoarthritis and also psoriasis.[9] Enthesitis related arthritis accounts for 5-10% of JIA patients and is an undifferentiated spondyloarthritis. Most patients are HLA-B27 positive, which is also related to more active joints involved.[10]

Many features have not been included in the ILAR classification and are subject of discussion.[11] Some of these parameters are: age at onset, detailed description of arthritis (smaller or larger joints/ symmetric or asymmetric joint involvement), total number of joints affected, presence of ANA,[12-14] presence of (chronic) anterior uveitis and (family history of) psoriasis.[15;16] In the near future genetic, immunologic, genome wide mRNA-expression and proteomic studies might reveal parameters that could also be incorporated into the JIA classification or even lead to a novel classification. As described, the classification of chronic arthritis in children is still evolving. The ultimate goal is to determine biologically distinct subtypes with a predictable response to treatment and outcome. However this situation is still far from being realized.

Table 1. The ILAR classification of the different categories (or subtypes) of JIA[6]

Subtype		Definition	Exclusion criteria*	Distribution#
Systemic JIA		Arthritis in one or more joints with or preceded by fever of at least 2 weeks' duration that is documented to be daily ("quotidian") for at least 3 days, and accompanied by one or more of the following: 1. Evanescent (nonfixed) erythematous rash 2. Generalized lymph node enlargement 3. Hepatomegaly and/or splenomegaly 4. Serositis	a,b,c,d	10-15%
Oligoarthritis	Persistent oligoarthritis	Arthritis affecting one to 4 joints during the first 6 months of disease and affecting not more than 4 joints throughout the disease course	a,b,c,d,e	50%
	Extended oligoarthritis	Arthritis affecting one to 4 joints during the first 6 months of disease and affecting a total of more than 4 joints after the first 6 months of disease	a,b,c,d,e	
RF-negative polyarthritis		Arthritis affecting 5 or more joints during the first 6 months of disease; test for RF is negative	a,b,c,d,e	20%
RF-positive polyarthritis		Arthritis affecting 5 or more joints during the first 6 months of disease; 2 or more tests for RF at least 3 months apart during the first 6 months of disease are positive	a,b,c,e	5%
Psoriatic arthritis		Arthritis and psoriasis, or arthritis and at least 2 of the following: 1. Dactylitis 2. Nail pitting or onycholysis 3. Psoriasis in a first-degree relative	b,c,d,e	5-10%
Enthesitis related arthritis		Arthritis and enthesitis, or arthritis or enthesitis with at least 2 of the following: 1. The presence of or a history of sacroiliac joint tenderness and/or inflammatory lumbosacral pain 2. The presence of HLA-B27 antigen 3. Onset of arthritis in a male over 6 years of age 4. Acute (symptomatic) anterior uveitis 5. History of ankylosing spondylitis, enthesitis related arthritis, sacroiliitis with inflammatory bowel disease, Reiter's syndrome, or acute anterior uveitis in a first-degree relative	a,d,e	5-10%
Undifferentiated arthritis		Arthritis that fulfills criteria in no category or in 2 or more of the above categories.		

RF: rheumatoid factor

*) Exclusion criteria:

a) Psoriasis or a history of psoriasis in the patient or first degree relative.

b) Arthritis in an HLA-B27 positive male beginning after the 6th birthday.

c) Ankylosing spondylitis, enthesitis related arthritis, sacroiliitis with inflammatory bowel disease, Reiter's syndrome, or acute anterior uveitis, or a history of one of these disorders in a first-degree relative.

d) The presence of IgM rheumatoid factor on at least 2 occasions at least 3 months apart.

e) The presence of systemic JIA in the patient.

#) Distribution in patients with a Caucasian European ethnicity

Prevalence of JIA

Worldwide the prevalence of JIA has been variably described, with a prevalence varying between 15/100.000 and 150/100.000.[17] Prevalence seems to be higher in the more northern countries of the northern hemisphere, as compared to countries lying closer to the equator. Remarkable is a difference in the distribution of subtypes; in the "Western world" the oligoarticular subtypes are the most frequent, whereas polyarthritis predominates in countries such as India, New Zealand and South Africa.[18] It should be noted this variation could be explained by methodological differences (like diagnostic difficulty of JIA, change in diagnostic criteria over time and variation in study design) or accessibility to health care. However this difference in prevalence of JIA subtypes might well be caused by differences in genetic background. Even when corrected for geographical differences, the genetic background (or ethnicity) is still related to the distribution of JIA subtype.[19] At this moment worldwide research is in progress to collect more data concerning the epidemiology of JIA (EPOCA study).[20]

Course of disease in JIA

Remission

Until 2004 each reported study of remission rates in JIA used a different definition of remission, making comparison of clinical outcome and efficacy of treatment difficult. An international consensus project has attempted to develop a definition of clinical remission and inactive disease. This project is still on-going.[21-23] The criteria for clinical inactive disease in oligoarthritis (persistent and extended), polyarthritis (RF negative and positive) and systemic JIA are: no joints with active disease, no fever, rash, serositis, splenomegaly or generalized lymphadenopathy attributable to JIA, no active uveitis (defined by the SUN Working Group), ESR or CRP level within normal limits or if elevated not attributable to JIA, physician's global assessment of disease activity score of best possible on the scale used and duration of morning stiffness <= 15 minutes.[22] The preliminary definition of clinical remission on medication is met when clinical inactive disease is present for a minimum of six continuous months with the patient on medication. Clinical remission is defined as clinical inactive disease that is present for a minimum of 12 continuous months with no use of medication.[21] Several studies using these definitions demonstrated only a small percentage of patients (approximately 30%) reaching clinical remission and a large percentage of patients with persistent active arthritis.[24;25] Consistently observed in different studies is that patients with persistent oligoarthritis have a more favorable outcome with a higher percentage of clinical remission and inactive disease (but varying from 43-84%).[24-27] The course of disease in the persistent oligoarthritis subtype is less progressive compared to both ex-

tended oligoarthritis and RF-negative polyarthritis. Patients with polyarticular JIA, and especially RF-positive polyarthritis, have a more progressive course with less remission and inactive disease.[24-26;28] Likewise, a recent prospective study with a follow-up of 17 years showed a large variability in disease course between the different subtypes, with remission overall present in only 40% of the JIA patients.[29]

Pattern of disease activity

The course of disease in JIA follows an unpredictable pattern of episodes with different levels of active disease and episodes of disease quiescence. This pattern of disease activity was described in more detail for the first time by Wallace et al,[30] who also introduced the term "cumulative time spent in a state of active or inactive disease". This outcome measure is the only one that takes the fluctuating character of disease activity in JIA into account. Patients with persistent oligoarthritis spend almost 60% of the time in a state of inactive disease, whereas patients with extended oligoarthritis or RF-negative polyarthritis have inactive disease only 30-36% of their time.[28;30] These outcomes are consistent with the higher percentage of continuous active disease described in extended oligoarthritis and RF-negative polyarthritis compared to persistent oligoarthritis.[27]

The aim of treatment is achieving a total absence of disease activity. However in patients with clinical remission, a biological disturbance is still present.[31;32] Different biomarkers (such as phagocyte activation marker S100A12 and myeloid-related protein MRP8/14) are under investigation for their role in defining immunological quiescence of JIA.[33]

Radiological damage

Many different scoring systems for radiological assessment of damage of the joints have been used in JIA; the Steinbrocker score (the most original radiologic score in RA), the Sharp/van der Heijde score and the Poznanski score.[34-36] Because of the lack of scoring systems for JIA patients, the Dijkstra composite score has been developed to describe radiological features of patients with JIA in a standardized manner.[37;38] Also specific for JIA is the Juvenile Arthritis Damage Index (JADI) that has been developed for articular (JADI-A) and extra-articular damage (JADI-E). This measure uses information obtained by physical examination and by a brief review of the patient's clinical history.[39] This scoring system correlates with radiological damage.[39] Most recently, a MRI scoring system (JAMRIS) for evaluating disease activity in the knee has been developed.[40]

Due to these different scoring systems and various study designs, the percentage of patients with radiological damage varies widely between 12% and 60%.[41-44] The

RF-positive polyarticular JIA patients have the most articular damage. A prolonged disease activity is associated with more radiologic damage, underlining the need of aggressive treatment inducing clinical remission at an early stage.[41;44;45]

JIA- associated uveitis

The most common extra-articular manifestation in JIA, is JIA-associated uveitis or chronic anterior uveitis.[46] It is also called silent uveitis because of the lack of symptoms, until complications cause symptoms of visual loss. This type of uveitis is associated with oligoarthritis, RF-negative polyarthritis and psoriatic arthritis, but is especially related to ANA positive, early-onset oligoarthritis (10-30%).[46-49] JIA-associated uveitis mostly develops during the first years after diagnosis of JIA, but can also precede the symptoms of arthritis or develop only many years after the JIA onset. Guidelines for ophthalmologic screening in patients with JIA have been formulated, taking the different risk factors into account.[50-52]

The course of JIA-associated uveitis is fluctuating and not always related to the activity level of arthritis. Uveitis that is already present at first screening and most likely preceded symptoms of arthritis, together with sustained inflammation of the eye are factors associated with complications and visual loss. Several complications of uveitis can be seen; cataract, band keratopathy, posterior synechiae, glaucoma, ocular hypotonia and macular edema. Recent studies reported complications, mainly cataract, in 15-20% of the uveitis patients and a few patients developed blindness. Both frequent screening and an altered therapeutic regime, with more aggressive medication, has led to a decrease in complication rate and a better outcome.[53-55]

Treatment of JIA-associated uveitis is based on a step-up approach and consists of topical or/and systemic glucocorticoids, followed by systemic methotrexate or azathioprine. When treatment fails adalimumab, infliximab, cyclosporin A or abatacept are administered as last step.[56;57]

Treatment of JIA

In the last decades, the treatment of JIA has changed dramatically. In 1981treatment of JIA was dominated by salicylate and non-steroidal anti-inflammatory drugs (NSAIDs). Treatment modalities such as gold, antimalarials or penicillamine were used in progressive polyarthritis and glucocorticoids only in selected patients with ongoing disease.[58]

The latest JIA treatment recommendations of the American College of Rheumatology, dating from 2011 (with an update concerning systemic JIA in 2013), are focused on disease modifying anti-rheumatic drugs (DMARDs) like methotrexate (MTX) and TNF-alpha-inhibitors for treating polyarticular JIA in an early stage as well as treating oligoarthritis following the use of intra-articular steroids (IAS) or NSAIDs.[59;60] The use of

IAS still has an important role in the treatment of oligoarthritis, however the long term benefits seem limited.[61]

DMARDs

In JIA the most frequently used non-biological DMARD is MTX, followed by Sulfasalazine. The biological DMARD that is mainly used in JIA is the TNF-alpha-inhibitor etanercept.

Methotrexate

Methotrexate (MTX) has an anti-proliferative effect, by acting as a folate antagonist interfering in (amongst others) purine and pyrimidine biosynthesis. It is also involved in an increased adenosine release, that has an immune-suppressive effect,[62] and acts as an inhibitor of cytokine production induced by T-cell activation.[63] Randomized controlled trials have proven the efficacy of a weekly low dose of MTX (10 mg/m^2) in the different subtypes of JIA (polyarticular JIA,[64] systemic JIA and extended oligoarthritis[65]). It is described that in recent years about 75% of all patients with JIA have used MTX during the first year of treatment.[66,67] When remission is reached, the time to withdrawal of MTX doesn't seem to have an influence on the relapse-rate or time to relapse.[68] No evidence-based guidelines for withdrawing MTX are available. MTX will be discussed in more detail in the General Discussion (Pharmacogenetics) section.

Etanercept

TNF-alpha is a pro-inflammatory cytokine that plays a (major) role in the pathogenesis of JIA. Soluble TNF-alpha-receptor acts as an inhibitor by binding TNF-alpha. Etanercept (Enbrel) is a fully human dimeric fusion protein consisting of the extracellular portion of the human TNF-alpha-receptor linked to the Fc portion of human lgG1. Clinical studies have first been performed in RA and thereafter the efficacy and safety has been investigated in polyarticular JIA in a double-blind study.[69] The dosage of etanercept is 0.4 mg per kilogram of body weight and it is given subcutaneously by injection twice weekly or at 0.8 mg per kilogram once weekly. Combining etanercept with prevailing DMARDs leads to a good response,[70;71] whereas etanercept as mono-treatment seems to be less effective.[72] In recent years, applying etanercept early in the disease course is not common, as only 5% of the patients have started in the first year after diagnosis.[66] Besides the effective use in polyarthritis, with a response to treatment in 74% of the patients,[69] etanercept results in a good response in extended oligoarthritis as well with a response defined by ACR 30 in 88.6% of the patients.[73] Although not registered for persistent oligoarthritis, it seems a justifiable option when treatment with IAS and MTX has failed.[74] Long-term efficacy (up to 8 years) and safety has been described in JIA.[75-80] Different registries have been established to monitor the efficacy and side

effects of treatment with etanercept in patients with JIA (German[76]; Dutch[79]; British[81] and Polish registry[82]). Several factors have been proposed as prognostic factors for a good response; a lower baseline disability score, the use of fewer DMARDs prior to initiating etanercept and a younger age at onset.[83;84] Successful withdrawal from etanercept has been described,[85] but also early flares in a substantial proportion of patients after discontinuation of treatment.[86] Several side effects have been suggested, such as infectious disease (for example tuberculosis), malignancies and the development of inflammatory bowel disease. However the relation with etanercept has not been clarified.

Other TNF-alpha-inhibitors such as adalimumab (Humira), a (humanized) monoclonal antibody against TNF-alpha and Infliximab (Remicade), a (human-mouse) chimeric monoclonal antibody against TNF-alpha, are less frequently used in JIA. Adalimumab especially seems to be the TNF-alpha-inhibitor of choice when JIA-associated uveitis is present.[87] Infliximab is used in high dosage in case of refractory or highly active arthritis and seems to be effective without serious side effects (most importantly infections) or infusion reactions.[88] Long-term data concerning the use of infliximab or adalimumab are lacking.[89]

Other biologic DMARDs

In JIA subtypes, besides systemic JIA, biologic DMARDs (or biologicals) other than TNF-alpha inhibitors, are not frequently used. One of the biologicals sometimes used in polyarticular JIA is abatacept. Abatacept is a recombinant fusion protein comprising the extracellular part of human CTLA-4 connected to a modified Fc part of IgG-1. It selectively modulates the T-cell co-stimulation, inhibiting the T-cell activation. It is proven effective in JIA patients not responding to previous treatment.[90;91] Tocilizumab, an interleukin-6 receptor inhibitor, has recently been proven effective and safe for treating patients with polyarticular JIA who failed to respond to MTX.[92]

In clinical practice, a large variability is observed in the treatment of patients with different subtypes of JIA. Various approaches for treating polyarticular JIA can be differentiated, such as a step up approach (non-biologic DMARD followed by a biologic DMARD), non-biologic and biologic DMARD combined, and direct use of a biologic DMARD.[93] The use of an early aggressive treatment (for example combining MTX and anti-TNF-alpha plus glucocorticoids versus MTX alone) seems to be more beneficial.[94;95] Also a large variation in treating mono-arthritis has been described, varying from IAS alone, a combination of IAS and NSAIDs or even NSAIDs with non-biological DMARD (mainly MTX).[96] This illustrates that the choice of best treatment has still not been resolved. An ability to better predict the course of disease and the response to treatment

would be helpful in guiding the pediatric rheumatologist in his/her decision about the best individual treatment.

Prognostic factors

In the last decades numerous studies have been performed to identify prognostic factors for the outcome of JIA. Outcome has been defined in different ways; as joint erosions, persistent disease and physical disability. Many factors have been suggested as prognostic factor: time to diagnosis, young age at onset, female sex, large number of affected joints, greater severity of arthritis or higher disability at onset (C-HAQ), symmetric disease, hip/wrist involvement, long duration of elevated erythrocyte sedimentation rate or inflammatory markers and a positive RF.[29;97-101] Of all these factors, only the different JIA subtypes have consistently been associated with a different rate of remission and can be regarded as having some prognostic value. Also a prolonged active disease in the first 6- 12 months seems to be associated with a worse long-term outcome.[29;98] However this is not a predictive factor that is present at disease onset. The aim of treatment is to reduce the time with active disease, as early in the disease course as possible. Prognostic factors or a predictive model that can be determined at disease onset are still needed.

Etiology

JIA is thought to be an autoimmune disease, although the pathogenesis remains largely unknown.[102-104] The initial trigger is supposed to be a self-antigen, that has not been identified, but cartilage-derived autoantigens (like aggrecan, fibrillin and MMP3) might be involved.[105] Following an autoreactive trigger, different parts of the immune response are activated such as (antigen-specific) T-cell response (T-helper-1/ T-helper-17 cells), activation of neutrophils, expansion of regulatory T-cells and the release of cytokines and chemokines. In JIA, as well as in other autoimmune diseases, the possible role of regulatory T-cells has been described.[106;107] Regulatory T-cells are supposed to maintain self-tolerance and suppress inflammation. Interleukin (IL)-2 plays an important role in the development and function of regulatory T-cells through its interaction with CD25 (IL2-receptor-alpha). At the site of inflammation increased levels of regulatory T-cells are consistently reported, although their exact contribution in the local inflammatory environment remains unclear.[108] Besides regulatory T-cells, also T-helper-17 cells have been reported to play a central role in the initiation and maintenance of the autoimmune reaction in JIA.[109;110] T-helper-17 cells produce the pro-inflammatory cytokines IL17 and IL22. An imbalance between pro-inflammatory T-helper-1/T-helper-17 cells and anti-inflammatory regulatory T-cells seems to be essential for developing JIA. The

persistent inflammation of the synovium eventually leads to cartilage destruction and bone erosions.
Systemic JIA has similarities with autoinflammatory syndromes. An overproduction of IL6 is present and patients have a unique IL1 signature. Treatment with tocilizumab (a humanized recombinant anti-IL6 receptor antibody) has been successful[111;112] and clinical trials show effect of IL1 blockade.[113]

Complex disease

JIA is thought to be a complex disease, in which interaction of environmental factors and multiple genetic factors exists.[114]

Environmental factors

A causal association between environmental factors and the development of JIA remains difficult. The trigger of the (auto)immune reaction might be a (viral) infection. Seasonal variation, suggesting an infectious etiology, is described only in systemic JIA, with a higher incidence in spring and summer. Stressful life events, physiological factors, perinatal factors, several viral and bacterial (such as streptococcal) infections have all been related to some degree to JIA and described in detail.[17]

Genetic factors

Familiar risk

A higher occurrence and similar clinical features of disease in affected siblings and families are suggestive for a genetic trait. As early as 1939 juvenile arthritis has been described in monozygotic twins.[115] Most of the (monozygotic) twins and affected sib pairs that have been studied have a concordance for disease subtype and course of disease.[116-120] Clinical features of juvenile arthritis are the same in familial cases and sporadic cases.[120;121] The relative risk (lambda) for first-degree family members has been estimated in small study cohorts to be 15-30.[114;117;118] Recently the relative risk was calculated using a large cohort of JIA patients combined with data from a population database and showed a relative risk in siblings of 11.6, declining to 5.8 in first cousins.[122] The risk for JIA attributable to familial factors was about 13%. These data strengthen the hypothesis that genetic factors play a role in the pathogenesis of JIA.

Ethnicity and gender

Differences in phenotypes of JIA are observed in different ethnic groups. African and Asian patients are more likely to have polyarticular JIA with a higher incidence of a positive RF.[123;124] In Caucasian patients (European and North American) the oligoarticu-

lar subtype is more common. Oligoarticular JIA with positive ANA and JIA-associated uveitis is even more rare in the non-Caucasian population.[125] As in other autoimmune diseases, a female preponderance is seen in JIA, with a female:male ratio of 7:1.

Clustering of autoimmune diseases in JIA families

Clustering of multiple autoimmune diseases in one family, and even in one patient, has been known for many years. The pattern of clustering of the 5 major autoimmune diseases, amongst which is RA, has been described and reviewed in detail.[126;127] Several studies have described the familial autoimmunity in JIA families. All studies indicate a higher prevalence of autoimmunity in JIA families compared to controls.[128-131]

Genetic association studies

The aim of genetic studies is to identify associations between genetic variations (like a single nucleotide polymorphism (SNP), microsatellite, variable number tandem repeat, insertions/deletions) and phenotypic traits. In order to establish a genetic association, different types of studies can be performed.[132]

In a family based study (the transmission disequilibrium test (TDT)), the transmission of a genetic marker from parents to their offspring is studied. It is expected that when a genetic variation is associated with disease, the transmission of that variation to affected offspring is more frequent than expected by Mendelian laws. In this type of study, the potential confounding effect of population stratification is avoided because parents act as controls for the cases (affected offspring). To generate enough power to detect associations with only small effects, the same number of cases is needed as in a case-control study, together with DNA of both parents.

In a case-control association study the difference in frequency of a genetic marker is studied between cases with a specific trait and controls lacking this trait. If a genetic marker is significantly more/less frequent in cases, than there is an association with the specific trait. When the genetic marker is the causal variant, a direct association is revealed. However most of the time a genetic marker is not the causal variant, but is in linkage disequilibrium (LD) with nearby (and possible causal) variants situated in one LD block (that is located between two recombination hot spots) and is called an indirect association. In case SNPs have been studied, the genetic marker of such an indirect association is called a tag SNP, representing an LD block. In case-control studies admixture of the population (population stratification) should be avoided, because it can cause false positive or false negative associations.

Different types of case-control association studies can be performed. In a candidate gene study the selected genetic markers are located in genes of specific interest and are thought to play a role in the mechanism that is investigated. In studies focusing

on the susceptibility of disease, genes are selected that might be involved in the pathogenesis and in studies that focus on the severity of disease, genes can be selected from pathways involved in ongoing inflammation. In genome wide association studies (GWAS) multiple genetic markers (covering a small fraction of the total sequence variation) are studied without underlying hypothesis, which could result in discovery of totally new and unexpected associations. Unfortunately a large cohort of cases and controls is needed to be able to correct for multiple testing. Nowadays the Human Genome Project has identified about 10 million common SNPs. Because of the LD, it is estimated that about 300,000 to 600,000 (tag)SNPs will cover the genetic variation between individuals.[133]

The aim of genetic association studies is to identify a genetic association, replicate/validate this association in an independent cohort and examine the functional consequence of this genetic marker (or associated causal variant). Thereafter the role in pathogenesis of disease (leading to better diagnosis and classification) or possible therapeutic consequences may be investigated.

Genetics association studies in JIA

HLA

The first genetics factor that has been studied in JIA was the human leukocyte antigen (HLA), because of its major role in T-cell immunity and because it is polymorphic and easily typed.[134] There are common HLA associations in the overall JIA patient group, but also different patterns of HLA association in different JIA subgroups are described. These subgroups do not always follow the ILAR classification.[135;136] Nowadays many genetic factors have been studied, however HLA remains to have the strongest association in JIA.[137]

Non-HLA associations

From 1998 onwards multiple case-control candidate gene association studies have been performed in JIA in selected genes (like IL-6, TNF-alpha, IL-1, IL-10, MIF). In most of these studies only a small cohort was available for study (median of 130 cases vs 276 controls)[138] and, therefore, these studies were underpowered to detect associations with a small effect size. The positive associations that were detected were often difficult to replicate. Systematic review of these studies identified that about 100 loci had been investigated until 2008.[138] However at that point in time (which was also the starting point of our study) only 5 associations were independently confirmed; PTPN22, MIF, SLC11A1 (NRAMP1), TNFA, and WISP3.

In recent years more loci associated with JIA have been replicated ; STAT4, TNFAIP3, IL2RA, TRAF1/C5, and VTCN1.[139] In addition to candidate gene studies, genome wide association studies (GWAS) have also been performed in JIA. The first GWAS revealed (besides HLA) a new association; VTCN1.[140] VTCN1 was discovered and replicated in an independent cohort. The GWAS published in 2012 revealed 3q13 as a novel susceptibility locus for JIA.[141] The most recent study using large scale genotyping focused on different regions of interest using dense genotyping on an Immunochip. This study revealed 14 new loci of interest.[137]
PTPN22 is a good example of an association in JIA that has been replicated in multiple independent cohorts (in both candidate gene studies and GWAS) and this will therefore be discussed in more detail.

PTPN22

PTPN22 encodes lymphoid-specific phosphatase, which is an inhibitor of the T-cell activation, preventing spontaneous activation and restricting the response to antigen. A functional SNP was discovered in codon 620, changing arginine into tryptophan, leading to a more efficient inhibition of T-cell activation that could have a part in failure to delete autoreactive T-cells during thymic selection or insufficient activity of regulatory T-cells.[142] In a candidate gene approach the association of this SNP was tested in diabetes mellitus type 1 (DM1) and a positive association of the tryptophan variant (allele 1858T) was found and replicated in an independent cohort.[143] Functional studies show that only lymphoid-specific phosphatase with Arg620 (allele 1858C) forms a complex with the C-terminal Src kinase (CSK), whereas lymphoid-specific phosphatase with Trp620 (allele 1858T) does not bind to CSK, resulting in a reduced T-cell receptor-mediated signaling.[144]
When the candidate gene study in DM1 was performed, a large genome wide association study of putative functional SNPs in RA was simultaneously conducted,[145] identifying PTPN22 as a risk factor for RA. PTPN22 (allele 1858) has since then been found to be associated with multiple autoimmune diseases such as SLE, celiac disease, Crohn's disease and thyroid autoimmunity. A positive association with JIA was first described in 2005[146] followed by mostly positive associations in several cohort studies (some of them with different ethnicities).[147-150] In the first genome wide association study in JIA, the association with PTPN22 was only modest, most likely because the SNPs that had been genotyped, had only low correlation with the causal variant.[140]
The precise implication of this altered function of lymphoid-specific phosphatase is still under investigation.[151;152] Lymphoid-specific phosphatase is currently being investigated as a possible drug target for treatment of autoimmunity.[144;153]

Common autoimmune susceptibility loci

Because of the familial clustering of autoimmune diseases it has always been thought that shared immunological pathways exist. When data from GWAS of multiple autoimmune diseases were compared (not including JIA), a large overlap in genetic susceptibility became clear.[154] Distinct shared immunological pathways were revealed; T-cell differentiation (e.g. IL10, IL18RAP, STAT3, STAT4, IL2RA), immune-cell signaling (e.g. CTLA4, PTPN22) and innate immunological response and TNF-alpha-signaling (e.g. TRAF/C5, TNFAIP3). Shared susceptibility genes encoding cytokines and chemokines were consistently found, together with shared loci with an (as yet) unknown function, that might include transcription factor binding sites.[155]

THIS THESIS

The aim of this thesis is to identify on the one hand non-HLA genetic factors that are associated with the susceptibility to develop JIA (part A) and additionally identify clinical and genetic factors that are associated with the differences in the course of disease and the response to treatment (part B).

In order to address these questions, a new independent cohort of Caucasian JIA patient has been created through collaboration with multiple rheumatology referral centers in North-Western Europe. Both DNA and detailed data on the course of disease and use of medication have been collected.

Several case-control candidate gene association studies have been performed in this new cohort of JIA patients comparing them to a population of healthy controls. Some of these studies only tested one hypothesis, such as TRAF1/C5 (Chapter 2) and the 4q27 locus (Chapter 3), based on the latest (at that time) discovered associations in RA (which of all the autoimmune diseases mostly resembles JIA). In Chapter 4 large scale genotyping is described, involving genes/ loci that have already been associated with JIA (in order to replicate associations) or other autoimmune diseases (in order to identify common autoimmune susceptibility loci that also concern JIA) or are involved in immune-regulation (in order to discover new associations).

To capture the fluctuating pattern of different levels of disease activity during the course of JIA, the parameter "percentage of active disease (in the first two years after disease onset)" has been used to define the course of disease. Both clinical (Chapter 5) and genetic factors (Chapter 6) have been studied in relation to the course of disease. Due to the fact that MTX is the most used DMARD in JIA, clinical and genetic factors associated with the response to MTX are of major interest and are explored in Chapter 7.

Associations with the susceptibility and the severity of JIA might reveal pathways involved in the pathogenesis of JIA and could bring to light important lead points for treatment of JIA. Clinical and genetic factors that are associated with the course of disease or the response to treatment could act as predictive parameters, alone or combined in a predictive model.

REFERENCES

(1) Hofer M, Southwood TR. Classification of childhood arthritis. *Best Pract Res Clin Rheumatol* 2002; 16(3):379-396.

(2) Prieur AM. What's in a name? Nomenclature of juvenile arthritis. A European view. *J Rheumatol Suppl* 1993; 40:9-11.

(3) Cassidy JT. What's in a name? Nomenclature of juvenile arthritis. A North American view. *J Rheumatol Suppl* 1993; 40:4-8.

(4) Fink CW. Proposal for the development of classification criteria for idiopathic arthritides of childhood. *J Rheumatol* 1995; 22(8):1566-1569.

(5) Petty RE. Classification of childhood arthritis: a work in progress. *Baillieres Clin Rheumatol* 1998; 12(2):181-190.

(6) Petty RE, Southwood TR, Manners P, Baum J, Glass DN, Goldenberg J et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. *J Rheumatol* 2004; 31(2):390-392.

(7) Martini A. Systemic juvenile idiopathic arthritis. *Autoimmun Rev* 2012; 12(1):56-59.

(8) van Rossum M, van Soesbergen R, de Kort S, ten Cate R, Zwinderman AH, de Jong B et al. Anti-cyclic citrullinated peptide (anti-CCP) antibodies in children with juvenile idiopathic arthritis. *J Rheumatol* 2003; 30(4):825-828.

(9) Stoll ML, Nigrovic PA. Subpopulations within juvenile psoriatic arthritis: a review of the literature. *Clin Dev Immunol* 2006; 13(2-4):377-380.

(10) Berntson L, Damgard M, Andersson-Gare B, Herlin T, Nielsen S, Nordal E et al. HLA-B27 predicts a more extended disease with increasing age at onset in boys with juvenile idiopathic arthritis. *J Rheumatol* 2008; 35(10):2055-2061.

(11) Martini A. It is time to rethink juvenile idiopathic arthritis classification and nomenclature. *Ann Rheum Dis* 2012; 71(9):1437-1439.

(12) Ravelli A, Felici E, Magni-Manzoni S, Pistorio A, Novarini C, Bozzola E et al. Patients with antinuclear antibody-positive juvenile idiopathic arthritis constitute a homogeneous subgroup irrespective of the course of joint disease. *Arthritis Rheum* 2005; 52(3):826-832.

(13) Ravelli A, Varnier GC, Oliveira S, Castell E, Arguedas O, Magnani A et al. Antinuclear antibody-positive patients should be grouped as a separate category in the classification of juvenile idiopathic arthritis. *Arthritis Rheum* 2011; 63(1):267-275.

(14) Nordal E, Songstad NT, Rygg M. Difficulties in defining antinuclear antibody-positive patients as a separate category in the classification of juvenile idiopathic arthritis: comment on the article by Ravelli et al. *Arthritis Rheum* 2011; 63(9):2835-2836.

(15) Butbul YA, Tyrrell PN, Schneider R, Dhillon S, Feldman BM, Laxer RM et al. Comparison of patients with juvenile psoriatic arthritis and nonpsoriatic juvenile idiopathic arthritis: how different are they? *J Rheumatol* 2009; 36(9):2033-2041.

(16) Merino R, de IJ, Garcia-Consuegra J. Evaluation of revised International League of Associations for Rheumatology classification criteria for juvenile idiopathic arthritis in Spanish children (Edmonton 2001). *J Rheumatol* 2005; 32(3):559-561.

(17) Berkun Y, Padeh S. Environmental factors and the geoepidemiology of juvenile idiopathic arthritis. *Autoimmun Rev* 2010; 9(5):A319-A324.

(18) Manners PJ, Bower C. Worldwide prevalence of juvenile arthritis why does it vary so much? *J Rheumatol* 2002; 29(7):1520-1530.

(19) Saurenmann RK, Rose JB, Tyrrell P, Feldman BM, Laxer RM, Schneider R et al. Epidemiology of juvenile idiopathic arthritis in a multiethnic cohort: ethnicity as a risk factor. *Arthritis Rheum* 2007; 56(6):1974-1984.

(20) Consolaro A, Ruperto N, Filocamo G, Lanni S, Bracciolini G, Garrone M et al. Seeking insights into the EPidemiology, treatment and Outcome of Childhood Arthritis through a multinational collaborative effort: Introduction of the EPOCA study. *Pediatr Rheumatol Online J* 2012; 10(1):39.

(21) Wallace CA, Ruperto N, Giannini E. Preliminary criteria for clinical remission for select categories of juvenile idiopathic arthritis. *J Rheumatol* 2004; 31(11):2290-2294.

(22) Wallace CA, Giannini EH, Huang B, Itert L, Ruperto N. American College of Rheumatology provisional criteria for defining clinical inactive disease in select categories of juvenile idiopathic arthritis. *Arthritis Care Res (Hoboken)* 2011; 63(7):929-936.

(23) Shenoi S, Wallace CA. Remission in juvenile idiopathic arthritis: current facts. *Curr Rheumatol Rep* 2010; 12(2):80-86.

(24) Lurati A, Salmaso A, Gerloni V, Gattinara M, Fantini F. Accuracy of Wallace criteria for clinical remission in juvenile idiopathic arthritis: a cohort study of 761 consecutive cases. *J Rheumatol* 2009; 36(7):1532-1535.

(25) Fernandes TA, Corrente JE, Magalhaes CS. Remission status follow-up in children with juvenile idiopathic arthritis. *J Pediatr (Rio J)* 2007; 83(2):141-148.

(26) Adib N, Silman A, Thomson W. Outcome following onset of juvenile idiopathic inflammatory arthritis: I. frequency of different outcomes. *Rheumatology (Oxford)* 2005; 44(8):995-1001.

(27) Nordal E, Zak M, Aalto K, Berntson L, Fasth A, Herlin T et al. Ongoing disease activity and changing categories in a long-term nordic cohort study of juvenile idiopathic arthritis. *Arthritis Rheum* 2011; 63(9):2809-2818.

(28) Ringold S, Seidel KD, Koepsell TD, Wallace CA. Inactive disease in polyarticular juvenile idiopathic arthritis: current patterns and associations. *Rheumatology (Oxford)* 2009; 48(8):972-977.

(29) Bertilsson L, Andersson-Gare B, Fasth A, Petersson IF, Forsblad-D'elia H. Disease course, outcome, and predictors of outcome in a population-based juvenile chronic arthritis cohort followed for 17 years. *J Rheumatol* 2013; 40(5):715-724.

(30) Wallace CA, Huang B, Bandeira M, Ravelli A, Giannini EH. Patterns of clinical remission in select categories of juvenile idiopathic arthritis. *Arthritis Rheum* 2005; 52(11):3554-3562.

(31) Prelog M, Schwarzenbrunner N, Tengg E, Sailer-Hock M, Kern H, Zimmerhackl LB et al. Quantitative alterations of CD8+ T cells in juvenile idiopathic arthritis patients in remission. *Clin Rheumatol* 2009; 28(4):385-389.

(32) Knowlton N, Jiang K, Frank MB, Aggarwal A, Wallace C, McKee R et al. The meaning of clinical remission in polyarticular juvenile idiopathic arthritis: gene expression profiling in peripheral blood mononuclear cells identifies distinct disease states. *Arthritis Rheum* 2009; 60(3):892-900.

(33) Gerss J, Roth J, Holzinger D, Ruperto N, Wittkowski H, Frosch M et al. Phagocyte-specific S100 proteins and high-sensitivity C reactive protein as biomarkers for a risk-adapted treatment to maintain remission in juvenile idiopathic arthritis: a comparative study. *Ann Rheum Dis* 2012; 71(12):1991-1997.

(34) Steinbrocker O, Traeger CH, Batterman RC. Therapeutic criteria in rheumatoid arthritis. *J Am Med Assoc* 1949; 140(8):659-662.

(35) van der Heijde D. How to read radiographs according to the Sharp/van der Heijde method. *J Rheumatol* 2000; 27(1):261-263.

(36) Poznanski AK, Hernandez RJ, Guire KE, Bereza UL, Garn SM. Carpal length in children--a useful measurement in the diagnosis of rheumatoid arthritis and some concenital malformation syndromes. *Radiology* 1978; 129(3):661-668.

(37) van Rossum MA, Zwinderman AH, Boers M, Dijkmans BA, van Soesbergen RM, Fiselier TJ et al. Radiologic features in juvenile idiopathic arthritis: a first step in the development of a standardized assessment method. *Arthritis Rheum* 2003; 48(2):507-515.

(38) van Rossum MA, Boers M, Zwinderman AH, van Soesbergen RM, Wieringa H, Fiselier TJ et al. Development of a standardized method of assessment of radiographs and radiographic change in juvenile idiopathic arthritis: introduction of the Dijkstra composite score. *Arthritis Rheum* 2005; 52(9):2865-2872.

(39) Viola S, Felici E, Magni-Manzoni S, Pistorio A, Buoncompagni A, Ruperto N et al. Development and validation of a clinical index for assessment of long-term damage in juvenile idiopathic arthritis. *Arthritis Rheum* 2005; 52(7):2092-2102.

(40) Hemke R, van Rossum MA, van Veenendaal M, Terra MP, Deurloo EE, de Jonge MC et al. Reliability and responsiveness of the Juvenile Arthritis MRI Scoring (JAMRIS) system for the knee. *Eur Radiol* 2013; 23(4):1075-1083.

(41) Solari N, Viola S, Pistorio A, Magni-Manzoni S, Vitale R, Ruperto N et al. Assessing current outcomes of juvenile idiopathic arthritis: a cross-sectional study in a tertiary center sample. *Arthritis Rheum* 2008; 59(11):1571-1579.

(42) Susic GZ, Stojanovic RM, Pejnovic NN, Damjanov NS, Soldatovic II, Jablanovic DB et al. Analysis of disease activity, functional disability and articular damage in patients with juvenile idiopathic arthritis: a prospective outcome study. *Clin Exp Rheumatol* 2011; 29(2):337-344.

(43) Bertilsson L, Andersson-Gare B, Fasth A, Forsblad-D'elia H. A 5-year prospective population-based study of juvenile chronic arthritis: onset, disease process, and outcome. *Scand J Rheumatol* 2012; 41(5):379-382.

(44) Lipinska J, Brozik H, Stanczyk J, Smolewska E. Anticitrullinated protein antibodies and radiological progression in juvenile idiopathic arthritis. *J Rheumatol* 2012; 39(5):1078-1087.

(45) Oen K, Reed M, Malleson PN, Cabral DA, Petty RE, Rosenberg AM et al. Radiologic outcome and its relationship to functional disability in juvenile rheumatoid arthritis. *J Rheumatol* 2003; 30(4):832-840.

(46) Kotaniemi K, Savolainen A, Karma A, Aho K. Recent advances in uveitis of juvenile idiopathic arthritis. *Surv Ophthalmol* 2003; 48(5):489-502.

(47) Rosenberg AM. Uveitis associated with childhood rheumatic diseases. *Curr Opin Rheumatol* 2002; 14(5):542-547.

(48) Foster CS. Diagnosis and treatment of juvenile idiopathic arthritis-associated uveitis. *Curr Opin Ophthalmol* 2003; 14(6):395-398.

(49) Twilt M, Swart vd Berg M, van Meurs JC, ten Cate R, van Suijlekom-Smit LW. Persisting uveitis antedating psoriasis in two boys. *Eur J Pediatr* 2003; 162(9):607-609.

(50) American Academy of Pediatrics Section on Rheumatology and Section on Ophthalmology: Guidelines for ophthalmologic examinations in children with juvenile rheumatoid arthritis. *Pediatrics* 1993; 92(2):295-296.

(51) Cassidy J, Kivlin J, Lindsley C, Nocton J. Ophthalmologic examinations in children with juvenile rheumatoid arthritis. *Pediatrics* 2006; 117(5):1843-1845.

(52) Heiligenhaus A, Niewerth M, Ganser G, Heinz C, Minden K. Prevalence and complications of uveitis in juvenile idiopathic arthritis in a population-based nation-wide study in Germany: suggested modification of the current screening guidelines. *Rheumatology (Oxford)* 2007; 46(6):1015-1019.

(53) Cabral DA, Petty RE, Malleson PN, Ensworth S, McCormick AQ, Shroeder ML. Visual prognosis in children with chronic anterior uveitis and arthritis. *J Rheumatol* 1994; 21(12):2370-2375.

(54) de Boer J, Wulffraat N, Rothova A. Visual loss in uveitis of childhood. *Br J Ophthalmol* 2003; 87(7):879-884.

(55) Chia A, Lee V, Graham EM, Edelsten C. Factors related to severe uveitis at diagnosis in children with juvenile idiopathic arthritis in a screening program. *Am J Ophthalmol* 2003; 135(6):757-762.

(56) Heiligenhaus A, Michels H, Schumacher C, Kopp I, Neudorf U, Niehues T et al. Evidence-based, interdisciplinary guidelines for anti-inflammatory treatment of uveitis associated with juvenile idiopathic arthritis. *Rheumatol Int* 2012; 32(5):1121-1133.

(57) Zulian F, Balzarin M, Falcini F, Martini G, Alessio M, Cimaz R et al. Abatacept for severe anti-tumor necrosis factor alpha refractory juvenile idiopathic arthritis-related uveitis. *Arthritis Care Res (Hoboken)* 2010; 62(6):821-825.

(58) Lindsley CB. Pharmacotherapy of juvenile rheumatoid arthritis. *Pediatr Clin North Am* 1981; 28(1):161-177.

(59) Beukelman T, Patkar NM, Saag KG, Tolleson-Rinehart S, Cron RQ, DeWitt EM et al. 2011 American College of Rheumatology recommendations for the treatment of juvenile idiopathic arthritis: initiation and safety monitoring of therapeutic agents for the treatment of arthritis and systemic features. *Arthritis Care Res (Hoboken)* 2011; 63(4):465-482.

(60) Ringold S, Weiss PF, Beukelman T, DeWitt EM, Ilowite NT, Kimura Y et al. 2013 update of the 2011 American College of Rheumatology recommendations for the treatment of juvenile idiopathic arthritis: recommendations for the medical therapy of children with systemic juvenile idiopathic arthritis and tuberculosis screening among children receiving biologic medications. *Arthritis Care Res (Hoboken)* 2013; 65(10):1551-1563.

(61) de Oliveira SJ, Albuquerque Pedrosa FT, Bicalho do NC, Corrente JE, Saad MC. Probability of remission of juvenile idiopathic arthritis following treatment with steroid joint injection. *Clin Exp Rheumatol* 2013.

(62) Cronstein BN, Naime D, Ostad E. The antiinflammatory mechanism of methotrexate. Increased adenosine release at inflamed sites diminishes leukocyte accumulation in an in vivo model of inflammation. *J Clin Invest* 1993; 92(6):2675-2682.

(63) Wessels JA, Huizinga TW, Guchelaar HJ. Recent insights in the pharmacological actions of methotrexate in the treatment of rheumatoid arthritis. *Rheumatology (Oxford)* 2008; 47(3):249-255.

(64) Giannini EH, Brewer EJ, Kuzmina N, Shaikov A, Maximov A, Vorontsov I et al. Methotrexate in resistant juvenile rheumatoid arthritis. Results of the U.S.A.-U.S.S.R. double-blind, placebo-controlled trial. The Pediatric Rheumatology Collaborative Study Group and The Cooperative Children's Study Group. *N Engl J Med* 1992; 326(16):1043-1049.

(65) Woo P, Southwood TR, Prieur AM, Dore CJ, Grainger J, David J et al. Randomized, placebo-controlled, crossover trial of low-dose oral methotrexate in children with extended oligoarticular or systemic arthritis. *Arthritis Rheum* 2000; 43(8):1849-1857.

(66) Pohjankoski H, Latva K, Kautiainen H, Saila H, Klaukka T, Virta L et al. First-year purchases of disease-modifying drugs of incident patients with chronic juvenile arthritis in Finland. *Clin Exp Rheumatol* 2011; 29(5):878-881.

(67) Beukelman T, Ringold S, Davis TE, DeWitt EM, Pelajo CF, Weiss PF et al. Disease-modifying antirheumatic drug use in the treatment of juvenile idiopathic arthritis: a cross-sectional analysis of the CARRA Registry. *J Rheumatol* 2012; 39(9):1867-1874.

(68) Foell D, Wulffraat N, Wedderburn LR, Wittkowski H, Frosch M, Gerss J et al. Methotrexate withdrawal at 6 vs 12 months in juvenile idiopathic arthritis in remission: a randomized clinical trial. *JAMA* 2010; 303(13):1266-1273.

(69) Lovell DJ, Giannini EH, Reiff A, Cawkwell GD, Silverman ED, Nocton JJ et al. Etanercept in children with polyarticular juvenile rheumatoid arthritis. Pediatric Rheumatology Collaborative Study Group. *N Engl J Med* 2000; 342(11):763-769.

(70) Schmeling H, Mathony K, John V, Keysser G, Burdach S, Horneff G. A combination of etanercept and methotrexate for the treatment of refractory juvenile idiopathic arthritis: a pilot study. *Ann Rheum Dis* 2001; 60(4):410-412.

(71) Haapasaari J, Kautiainen H, Hannula S, Pohjankoski H, Hakala M. Good results from combining etanercept to prevailing DMARD therapy in refractory juvenile idiopathic arthritis. *Clin Exp Rheumatol* 2002; 20(6):867-870.

(72) Horneff G, De Bock F, Foeldvari I, Girschick HJ, Michels H, Moebius D et al. Safety and efficacy of combination of etanercept and methotrexate compared to treatment with etanercept only in patients with juvenile idiopathic arthritis (JIA): preliminary data from the German JIA Registry. *Ann Rheum Dis* 2009; 68(4):519-525.

(73) Horneff G, Burgos-Vargas R, Constantin T, Foeldvari I, Vojinovic J, Chasnyk VG et al. Efficacy and safety of open-label etanercept on extended oligoarticular juvenile idiopathic arthritis, enthesitis-related arthritis and psoriatic arthritis: part 1 (week 12) of the CLIPPER study. *Ann Rheum Dis* 2013.

(74) Anink J, Otten MH, Prince FH, Hoppenreijs EP, Wulffraat NM, Swart JF et al. Tumour necrosis factor-blocking agents in persistent oligoarticular juvenile idiopathic arthritis: results from the Dutch Arthritis and Biologicals in Children Register. *Rheumatology (Oxford)* 2013; 52(4):712-717.

(75) Lovell DJ, Giannini EH, Reiff A, Jones OY, Schneider R, Olson JC et al. Long-term efficacy and safety of etanercept in children with polyarticular-course juvenile rheumatoid arthritis: interim results from an ongoing multicenter, open-label, extended-treatment trial. *Arthritis Rheum* 2003; 48(1):218-226.

(76) Horneff G, Schmeling H, Biedermann T, Foeldvari I, Ganser G, Girschick HJ et al. The German etanercept registry for treatment of juvenile idiopathic arthritis. *Ann Rheum Dis* 2004; 63(12):1638-1644.

(77) Lovell DJ, Reiff A, Jones OY, Schneider R, Nocton J, Stein LD et al. Long-term safety and efficacy of etanercept in children with polyarticular-course juvenile rheumatoid arthritis. *Arthritis Rheum* 2006; 54(6):1987-1994.

(78) Lovell DJ, Reiff A, Ilowite NT, Wallace CA, Chon Y, Lin SL et al. Safety and efficacy of up to eight years of continuous etanercept therapy in patients with juvenile rheumatoid arthritis. *Arthritis Rheum* 2008; 58(5):1496-1504.

(79) Prince FH, Twilt M, ten Cate R, van Rossum MA, Armbrust W, Hoppenreijs EP et al. Long-term follow-up on effectiveness and safety of etanercept in juvenile idiopathic arthritis: the Dutch national register. *Ann Rheum Dis* 2009; 68(5):635-641.

(80) Giannini EH, Ilowite NT, Lovell DJ, Wallace CA, Rabinovich CE, Reiff A et al. Long-term safety and effectiveness of etanercept in children with selected categories of juvenile idiopathic arthritis. *Arthritis Rheum* 2009; 60(9):2794-2804.

(81) Southwood TR, Foster HE, Davidson JE, Hyrich KL, Cotter CB, Wedderburn LR et al. Duration of etanercept treatment and reasons for discontinuation in a cohort of juvenile idiopathic arthritis patients. *Rheumatology (Oxford)* 2011; 50(1):189-195.

(82) Zuber Z, Rutkowska-Sak L, Postepski J, Dobrzyniecka B, Opoka-Winiarska V, Kobusinska K et al. Etanercept treatment in juvenile idiopathic arthritis: the Polish registry. *Med Sci Monit* 2011; 17(12):SR35-SR42.

(83) Otten MH, Prince FH, Armbrust W, ten Cate R, Hoppenreijs EP, Twilt M et al. Factors associated with treatment response to etanercept in juvenile idiopathic arthritis. *JAMA* 2011; 306(21):2340-2347.

(84) Solari N, Palmisani E, Consolaro A, Pistorio A, Viola S, Buoncompagni A et al. Factors associated with achievement of inactive disease in children with juvenile idiopathic arthritis treated with etanercept. *J Rheumatol* 2013; 40(2):192-200.

(85) Baszis K, Garbutt J, Toib D, Mao J, King A, White A et al. Clinical outcomes after withdrawal of anti-tumor necrosis factor alpha therapy in patients with juvenile idiopathic arthritis: a twelve-year experience. *Arthritis Rheum* 2011; 63(10):3163-3168.

(86) Postepski J, Kobusinska K, Olesinska E, Osinska V, Opoka-Winiarska V. Clinical remission in juvenile idiopathic arthritis after termination of etanercept. *Rheumatol Int* 2013; 33(10):2657-2660.

(87) Anink J, Otten MH, Gorter SL, Prince FH, van Rossum MA, van den Berg JM et al. Treatment choices of paediatric rheumatologists for juvenile idiopathic arthritis: etanercept or adalimumab? *Rheumatology (Oxford)* 2013; 52(9):1674-1679.

(88) Tambralli A, Beukelman T, Weiser P, Atkinson TP, Cron RQ, Stoll ML. High doses of infliximab in the management of juvenile idiopathic arthritis. *J Rheumatol* 2013; 40(10):1749-1755.

(89) Ungar WJ, Costa V, Burnett HF, Feldman BM, Laxer RM. The use of biologic response modifiers in polyarticular-course juvenile idiopathic arthritis: a systematic review. *Semin Arthritis Rheum* 2013; 42(6):597-618.

(90) Ruperto N, Lovell DJ, Quartier P, Paz E, Rubio-Perez N, Silva CA et al. Abatacept in children with juvenile idiopathic arthritis: a randomised, double-blind, placebo-controlled withdrawal trial. *Lancet* 2008; 372(9636):383-391.

(91) Ruperto N, Lovell DJ, Quartier P, Paz E, Rubio-Perez N, Silva CA et al. Long-term safety and efficacy of abatacept in children with juvenile idiopathic arthritis. *Arthritis Rheum* 2010; 62(6):1792-1802.

(92) Brunner H, Ruperto N, Zuber Z, Cuttica RJ, Xavier R, Calvo I et al. A4: Efficacy and Safety of Tocilizumab in Patients With Polyarticular-Course Juvenile Idiopathic Arthritis: 2-Year Data From CHERISH. *Arthritis Rheumatol* 2014; 66 Suppl 11:S5-S6.

(93) Ringold S, Weiss PF, Colbert RA, DeWitt EM, Lee T, Onel K et al. Childhood arthritis and rheumatology research alliance consensus treatment plans for new onset polyarticular juvenile idiopathic arthritis. *Arthritis Care Res (Hoboken)* 2014 Jul;66(7):1063-72

(94) Tynjala P, Vahasalo P, Tarkiainen M, Kroger L, Aalto K, Malin M et al. Aggressive combination drug therapy in very early polyarticular juvenile idiopathic arthritis (ACUTE-JIA): a multicentre randomised open-label clinical trial. *Ann Rheum Dis* 2011; 70(9):1605-1612.

(95) Wallace CA, Giannini EH, Spalding SJ, Hashkes PJ, O'Neil KM, Zeft AS et al. Trial of early aggressive therapy in polyarticular juvenile idiopathic arthritis. *Arthritis Rheum* 2012; 64(6):2012-2021.

(96) Beukelman T, Guevara JP, Albert DA, Sherry DD, Burnham JM. Variation in the initial treatment of knee monoarthritis in juvenile idiopathic arthritis: a survey of pediatric rheumatologists in the United States and Canada. *J Rheumatol* 2007; 34(9):1918-1924.

(97) Flato B, Lien G, Smerdel A, Vinje O, Dale K, Johnston V et al. Prognostic factors in juvenile rheumatoid arthritis: a case-control study revealing early predictors and outcome after 14.9 years. *J Rheumatol* 2003; 30(2):386-393.

(98) Ravelli A, Martini A. Early predictors of outcome in juvenile idiopathic arthritis. *Clin Exp Rheumatol* 2003; 21(5 Suppl 31):S89-S93.

(99) Adib N, Silman A, Thomson W. Outcome following onset of juvenile idiopathic inflammatory arthritis: II. predictors of outcome in juvenile arthritis. *Rheumatology (Oxford)* 2005; 44(8):1002-1007.

(100) Selvaag AM, Lien G, Sorskaar D, Vinje O, Forre O, Flato B. Early disease course and predictors of disability in juvenile rheumatoid arthritis and juvenile spondyloarthropathy: a 3 year prospective study. *J Rheumatol* 2005; 32(6):1122-1130.

(101) Oen K, Tucker L, Huber AM, Miettunen P, Scuccimarri R, Campillo S et al. Predictors of early inactive disease in a juvenile idiopathic arthritis cohort: results of a Canadian multicenter, prospective inception cohort study. *Arthritis Rheum* 2009; 61(8):1077-1086.

(102) Prakken BJ, Albani S. Using biology of disease to understand and guide therapy of JIA. *Best Pract Res Clin Rheumatol* 2009; 23(5):599-608.

(103) Prakken B, Albani S, Martini A. Juvenile idiopathic arthritis. *Lancet* 2011; 377(9783):2138-2149.

(104) Lin YT, Wang CT, Gershwin ME, Chiang BL. The pathogenesis of oligoarticular/polyarticular vs systemic juvenile idiopathic arthritis. *Autoimmun Rev* 2011; 10(8):482-489.

(105) Kamphuis S, Hrafnkelsdottir K, Klein MR, de Jager W, Haverkamp MH, van Bilsen JH et al. Novel self-epitopes derived from aggrecan, fibrillin, and matrix metalloproteinase-3 drive distinct autoreactive T-cell responses in juvenile idiopathic arthritis and in health. *Arthritis Res Ther* 2006; 8(6):R178.

(106) de Kleer IM, Wedderburn LR, Taams LS, Patel A, Varsani H, Klein M et al. CD4+CD25bright regulatory T cells actively regulate inflammation in the joints of patients with the remitting form of juvenile idiopathic arthritis. *J Immunol* 2004; 172(10):6435-6443.

(107) Kamphuis S, Kuis W, de Jager W, Teklenburg G, Massa M, Gordon G et al. Tolerogenic immune responses to novel T-cell epitopes from heat-shock protein 60 in juvenile idiopathic arthritis. *Lancet* 2005; 366(9479):50-56.

(108) Mijnheer G, Prakken BJ, van Wijk F. The effect of autoimmune arthritis treatment strategies on regulatory T-cell dynamics. *Curr Opin Rheumatol* 2013; 25(2):260-267.

(109) Nistala K, Moncrieffe H, Newton KR, Varsani H, Hunter P, Wedderburn LR. Interleukin-17-producing T cells are enriched in the joints of children with arthritis, but have a reciprocal relationship to regulatory T cell numbers. *Arthritis Rheum* 2008; 58(3):875-887.

(110) Hemdan NY, Birkenmeier G, Wichmann G, Abu El-Saad AM, Krieger T, Conrad K et al. Interleukin-17-producing T helper cells in autoimmunity. *Autoimmun Rev* 2010; 9(11):785-792.

(111) Yokota S, Miyamae T, Imagawa T, Katakura S, Kurosawa R, Mori M. Clinical study of tocilizumab in children with systemic-onset juvenile idiopathic arthritis. *Clin Rev Allergy Immunol* 2005; 28(3):231-238.

(112) Yokota S, Imagawa T, Mori M, Miyamae T, Aihara Y, Takei S et al. Efficacy and safety of tocilizumab in patients with systemic-onset juvenile idiopathic arthritis: a randomised, double-blind, placebo-controlled, withdrawal phase III trial. *Lancet* 2008; 371(9617):998-1006.

(113) Quartier P, Allantaz F, Cimaz R, Pillet P, Messiaen C, Bardin C et al. A multicentre, randomised, double-blind, placebo-controlled trial with the interleukin-1 receptor antagonist anakinra in patients with systemic-onset juvenile idiopathic arthritis (ANAJIS trial). *Ann Rheum Dis* 2011; 70(5):747-754.

(114) Glass DN, Giannini EH. Juvenile rheumatoid arthritis as a complex genetic trait. *Arthritis Rheum* 1999; 42(11):2261-2268.

(115) Baum J, Fink C. Juvenile rheumatoid arthritis in monozygotic twins: a case report and review of the literature. *Arthritis Rheum* 1968; 11(1):33-36.

(116) Moroldo MB, Tague BL, Shear ES, Glass DN, Giannini EH. Juvenile rheumatoid arthritis in affected sibpairs. *Arthritis Rheum* 1997; 40(11):1962-1966.

(117) Prahalad S, Ryan MH, Shear ES, Thompson SD, Glass DN, Giannini EH. Twins concordant for juvenile rheumatoid arthritis. *Arthritis Rheum* 2000; 43(11):2611-2612.

(118) Savolainen A, Saila H, Kotaniemi K, Kaipianen-Seppanen O, Leirisalo-Repo M, Aho K. Magnitude of the genetic component in juvenile idiopathic arthritis. *Ann Rheum Dis* 2000; 59(12):1001.

(119) Saila H, Savolainen A, Tuomilehto-Wolf E, Tuomilehto J, Leirisalo-Repo M. Type of onset of juvenile idiopathic arthritis in monozygotic twins can be phenotypically different. *J Rheumatol* 2000; 27(9):2289-2290.

(120) Moroldo MB, Chaudhari M, Shear E, Thompson SD, Glass DN, Giannini EH. Juvenile rheumatoid arthritis affected sibpairs: extent of clinical phenotype concordance. *Arthritis Rheum* 2004; 50(6):1928-1934.

(121) Saila HM, Savolainen HA, Kotaniemi KM, Kaipiainen-Seppanen OA, Leirisalo-Repo MT, Aho KV. Juvenile idiopathic arthritis in multicase families. *Clin Exp Rheumatol* 2001; 19(2):218-220.

(122) Prahalad S, Zeft AS, Pimentel R, Clifford B, McNally B, Mineau GP et al. Quantification of the familial contribution to juvenile idiopathic arthritis. *Arthritis Rheum* 2010; 62(8):2525-2529.

(123) Graham TB, Glass DN. Juvenile rheumatoid arthritis: ethnic differences in diagnostic types. *J Rheumatol* 1997; 24(9):1677-1679.

(124) Schwartz MM, Simpson P, Kerr KL, Jarvis JN. Juvenile rheumatoid arthritis in African Americans. *J Rheumatol* 1997; 24(9):1826-1829.

(125) Oen K. Comparative epidemiology of the rheumatic diseases in children. *Curr Opin Rheumatol* 2000; 12(5):410-414.

(126) Hemminki K, Li X, Sundquist J, Sundquist K. Familial associations of rheumatoid arthritis with autoimmune diseases and related conditions. *Arthritis Rheum* 2009; 60(3):661-668.

(127) Cardenas-Roldan J, Rojas-Villarraga A, Anaya JM. How do autoimmune diseases cluster in families? A systematic review and meta-analysis. *BMC Med* 2013; Mar 18;11:73.

(128) Pachman LM, Hayford JR, Hochberg MC, Pallansch MA, Chung A, Daugherty CD et al. New-onset juvenile dermatomyositis: comparisons with a healthy cohort and children with juvenile rheumatoid arthritis. *Arthritis Rheum* 1997; 40(8):1526-1533.

(129) Prahalad S, Shear ES, Thompson SD, Giannini EH, Glass DN. Increased prevalence of familial autoimmunity in simplex and multiplex families with juvenile rheumatoid arthritis. *Arthritis Rheum* 2002; 46(7):1851-1856.

(130) Huang CM, Yang YH, Chiang BL. Different familial association patterns of autoimmune diseases between juvenile-onset systemic lupus erythematosus and juvenile rheumatoid arthritis. *J Microbiol Immunol Infect* 2004; 37(2):88-94.

(131) Pohjankoski H, Kautiainen H, Kotaniemi K, Korppi M, Savolainen A. Diabetes, coeliac disease, multiple sclerosis and chronic arthritis in first-degree relatives of patients with juvenile idiopathic arthritis. *Acta Paediatr* 2012; 101(7):767-771.

(132) Cordell HJ, Clayton DG. Genetic association studies. *Lancet* 2005; 366(9491):1121-1131.

(133) Naidoo N, Pawitan Y, Soong R, Cooper DN, Ku CS. Human genetics and genomics a decade after the release of the draft sequence of the human genome. *Hum Genomics* 2011; 5(6):577-622.

(134) Forre O, Dobloug JH, Hoyeraal HM, Thorsby E. HLA antigens in juvenile arthritis. Genetic basis for the different subtypes. *Arthritis Rheum* 1983; 26(1):35-38.

(135) Thomson W, Barrett JH, Donn R, Pepper L, Kennedy LJ, Ollier WE et al. Juvenile idiopathic arthritis classified by the ILAR criteria: HLA associations in UK patients. *Rheumatology (Oxford)* 2002; 41(10):1183-1189.

(136) Hollenbach JA, Thompson SD, Bugawan TL, Ryan M, Sudman M, Marion M et al. Juvenile idiopathic arthritis and HLA class I and class II interactions and age-at-onset effects. *Arthritis Rheum* 2010; 62(6):1781-1791.

(137) Hinks A, Cobb J, Marion MC, Prahalad S, Sudman M, Bowes J et al. Dense genotyping of immune-related disease regions identifies 14 new susceptibility loci for juvenile idiopathic arthritis. *Nat Genet* 2013; 45(6):664-669.

(138) Prahalad S, Glass DN. A comprehensive review of the genetics of juvenile idiopathic arthritis. *Pediatr Rheumatol Online J* 2008 Jul 21;6:11

(139) Angeles-Han S, Prahalad S. The genetics of juvenile idiopathic arthritis: what is new in 2010? *Curr Rheumatol Rep* 2010; 12(2):87-93.

(140) Hinks A, Barton A, Shephard N, Eyre S, Bowes J, Cargill M et al. Identification of a novel susceptibility locus for juvenile idiopathic arthritis by genome-wide association analysis. *Arthritis Rheum* 2009; 60(1):258-263.

(141) Thompson SD, Marion MC, Sudman M, Ryan M, Tsoras M, Howard TD et al. Genome-wide association analysis of juvenile idiopathic arthritis identifies a new susceptibility locus at chromosomal region 3q13. *Arthritis Rheum* 2012; 64(8):2781-2791.

(142) Vang T, Congia M, Macis MD, Musumeci L, Orru V, Zavattari P et al. Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant. *Nat Genet* 2005; 37(12):1317-1319.

(143) Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M et al. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat Genet* 2004; 36(4):337-338.

(144) Vang T, Liu WH, Delacroix L, Wu S, Vasile S, Dahl R et al. LYP inhibits T-cell activation when dissociated from CSK. *Nat Chem Biol* 2012; 8(5):437-446.

(145) Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC et al. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am J Hum Genet* 2004; 75(2):330-337.

(146) Viken MK, Amundsen SS, Kvien TK, Boberg KM, Gilboe IM, Lilleby V et al. Association analysis of the 1858C>T polymorphism in the PTPN22 gene in juvenile idiopathic arthritis and other autoimmune diseases. *Genes Immun* 2005; 6(3):271-273.

(147) Hinks A, Barton A, John S, Bruce I, Hawkins C, Griffiths CE et al. Association between the PTPN22 gene and rheumatoid arthritis and juvenile idiopathic arthritis in a UK population: further support that PTPN22 is an autoimmunity gene. *Arthritis Rheum* 2005; 52(6):1694-1699.

(148) Ellis JA, Chavez RA, Pezic A, Ponsonby AL, Akikusa JD, Allen RC et al. Independent replication analysis of genetic loci with previous evidence of association with juvenile idiopathic arthritis. *Pediatr Rheumatol Online J* 2013; 11(1):12.

(149) Kaalla MJ, Broadaway KA, Rohani-Pichavant M, Conneely KN, Whiting A, Ponder L et al. Meta-analysis confirms association between TNFA-G238A variant and JIA, and between PTPN22-C1858T variant and oligoarticular, RF-polyarticular and RF-positive polyarticular JIA. *Pediatr Rheumatol Online J* 2013; 11(1):40.

(150) Dimopoulou DG, Zervou MI, Trachana M, Myrthianou E, Pratsidou-Gertsi P, Kardassis D et al. Investigation of juvenile idiopathic arthritis susceptibility loci: results from a Greek population. *Hum Immunol* 2013; 74(9):1194-1198.

(151) Fousteri G, Liossis SN, Battaglia M. Roles of the protein tyrosine phosphatase PTPN22 in immunity and autoimmunity. *Clin Immunol* 2013; 149(3):556-565.

(152) Bottini N, Peterson EJ. Tyrosine Phosphatase PTPN22: Multifunctional Regulator of Immune Signaling, Development, and Disease. *Annu Rev Immunol* 2014; 32:83-119.

(153) Du J, Qiao Y, Sun L, Wang X. Lymphoid-specific tyrosine phosphatase (Lyp): a potential drug target for treatment of autoimmune diseases. *Curr Drug Targets* 2014; 15(3):335-346.

(154) Zhernakova A, van Diemen CC, Wijmenga C. Detecting shared pathogenesis from the shared genetics of immune-related diseases. *Nat Rev Genet* 2009; 10(1):43-55.

(155) Maurano MT, Humbert R, Rynes E, Thurman RE, Haugen E, Wang H et al. Systematic localization of common disease-associated variation in regulatory DNA. *Science* 2012; 337(6099):1190-1195.