From Department of Medicine, Solna

Karolinska Institutet, Stockholm, Sweden

UNDERSTANDING JUVENILE IDIOPATHIC ARTHRITIS- A MULTIDIMENSIONAL APPROACH

Raya Saleh

Stockholm 2024

Understanding Juvenile Idiopathic Arthritis- a multidimensional approach

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Raya Saleh

Principal Supervisor:

Professor Helena Erlandsson Harris
Karolinska Institutet,
Department of Medicine Solna,
Division of Rheumatology,
Stockholm, Sweden

Co-supervisors:

Associate Professor Leonid Padyukov
Karolinska Institutet
Department of Medicine Solna,
Division of Rheumatology,
Stockholm, Sweden

Erik Sundberg, MD, PhD
Karolinska Institutet,
Department of Women's and Children's Health,
Division of Clinical Paediatrics,
Stockholm, Sweden

Opponent:
Professor Ellen Berit Nordal
UiT The Arctic University of
Norway, Faculty of Health
Sciences, Tromsø, Norway

Examination Board:

Professor Maja Jagodic
Karolinska Institutet,
Department of Clinical
Neuroscience, Stockholm, Sweden

Professor Liv Eidsmo
University of Copenhagen,
Leo Foundation Skin
Immunology Research Center,
Department of Immunology and
Microbiology, Copenhagen,
Denmark

Professor Göran Andersson
Swedish University of Agricultural
Sciences, Molecular Genetics and
Bioinformatics, Uppsala, Sweden

POPULAR SCIENCE SUMMARY OF THE THESIS

"*Cracking the Code of Juvenile Idiopathic Arthritis (JIA): Unraveling the Genetic and Immunological Mysteries*"

Juvenile Idiopathic Arthritis (JIA) is a complex, often misunderstood condition that affects children, causing painful inflammation in their joints. This thesis delves deep into the mysteries of this condition, seeking to shed light on its genetic and immunological roots.

Unveiling the Genetic Secrets:

The first part of the thesis embarks on a journey to decode the genetic factors underlying JIA. By examining the DNA of children with JIA, the study finds connections between specific genetic variations and the likelihood of developing JIA. This genetic detective work is a promising step towards more accurate diagnosis and tailored treatment options, offering hope to those affected by JIA in future.

Antibodies and Autoimmunity:

The thesis then takes a closer look at antibodies and the immune system. It uncovers the presence of specific antibodies called anti-citrullinated antibodies (ACPA) in children with JIA. While ACPAs are well-known in adults with Rheumatoid Arthritis, this study scrutinizes their occurrence in children with JIA, opening up new avenues for research into the immune aspects of the condition. Understanding the immune processes at play in JIA could pave the way for targeted therapies that may improve the lives of affected children.

Predicting Joint Damage:

The third part of the thesis explores the early signs of joint damage in JIA, a crucial piece of the puzzle to improving treatment and outcomes. By studying certain biomarkers in the blood and synovial fluid of affected children, this research offers potential tools for predicting and monitoring joint damage. These insights could lead to early interventions and more effective management of the condition.

What Lies Ahead:

In conclusion, the thesis's findings present new prospects for the future of JIA research and treatment. By delving into genetics, immune responses, and early disease indicators, it lays the groundwork for more precise and effective approaches to managing JIA and could contribute to better outcomes for affected children in future.

ABSTRACT

The overarching objective of this doctoral thesis is to enhance the understanding of Juvenile Idiopathic Arthritis (JIA), a complex autoimmune disease affecting children. With the aim of filling the knowledge gaps in JIA, we conducted a comprehensive study on biomarkers with distinct biological implications, including genetic variations, autoantibodies, and plasma and synovial fluid proteins, in individuals with JIA and controls.

The goal for Study I was to investigate the genetics of JIA by employing a case control genome-wide association analysis within a Swedish JIA cohort and controls. This study identified relations between specific Human Leukocyte Antigen (HLA) alleles and the presence of autoantibodies, namely anti-nuclear antibodies (ANA). We found that *HLA-DRB1-DQA1-DQB1* haplotype was strongly associated with increased JIA risk in the overall cohort which *HLA-DRB1*08* might be the primary allele linked to JIA susceptibility. We also found that the ANA-positive JIA has a stronger association with this haplotype. Further logistic analysis revealed that *HLA- DRB1*08* and *DRB1*11* alleles, female sex, and lower age at onset all contribute to ANA-positivity in JIA.

Study II explored the involvement of *LACC1* gene polymorphisms in non-systemic JIA for the first time. We detected significant associations of the *LACC1* gene polymorphisms with JIA as well as for inflammatory bowel disease (IBD) suggesting mutual mechanisms linking different autoimmune conditions.

The goal of study III was to examine the prevalence of antibodies against citrullinated proteins/peptides (ACPA) in the plasma of Swedish JIA patients and juvenile control cohort for the first time and investigate the genetic predispositions underpinning ACPA presence in

JIA. We also set to specify the ACPA reactivity profiles through utilization of a multiplex microarray platform, which facilitates the simultaneous detection of multiple reactivities. Anti-CCP antibodies were detected in 6.2% of JIA patients and 2% of controls and ACPAs with a variety of different reactivities were detected with high frequency in anti-CCP-positive patients as well as anti-CCP-negative patients although with a lower frequency. Anti-CCP antibodies correlated with rheumatoid factor, *HLA-DRB1* shared epitope, RF-positive polyarthritis subtype and older age at onset.

Our core focus in the Study IV was to assess levels of biomarkers for early joint degradation in JIA compared to healthy children or juveniles with knee injuries, extending the potential for early detection and tracking of joint damage but also for distinguishing of molecular signatures associated with joint destruction in JIA. We assessed triple-paired synovial fluid, plasma and urine samples from JIA patients compared to plasma samples from healthy children and synovial fluid from knee-injured juveniles. We found that plasma levels of ARGS, C2C, COMP, and TRAP5b were elevated in children JIA when compared to healthy children. When comparing JIA patients to juveniles with knee injuries, we observed increased levels of synovial fluid C2C and TRAP5b in JIA, but decreased levels of ARGS and COMP. Among JIA patients, we discovered positive correlations between local (synovial fluid) and systemic (plasma/urine) levels of bone biomarkers. Additionally, we observed negative correlations between age and plasma levels of C2C and TRAP5b. However, we did not find any correlations between these biomarkers and gender, the number of affected joints, disease duration, or medication use.

Our investigation of diverse biomarkers and potential mechanisms in JIA has demonstrates novel insights into the multifaceted pathological profile of the disease. These findings not only show relevance to clinical phenotypes but also transcend current disease classification

criteria. This thesis contributes to the advancement of our understanding of these intricate mechanisms, underscoring the pivotal roles of genetics and autoimmunity in the development of disease subtypes within JIA.

LIST OF SCIENTIFIC PAPERS

I. Genetic association of antinuclear antibodies with HLA in JIA patients
RAYA SALEH, Erik Sundberg, Katarina Tengvall, Lars Alfredsson, Ingrid
Kockum, Leonid Padyukov*, Helena Erlandsson Harris*
Manuscript
*These authors share last authorship

II. LACC1 polymorphisms in inflammatory bowel disease and juvenile
idiopathic arthritis
G Assadi, R SALEH, F Hadizadeh, L Vesterlund, F Bonfiglio, J Halfvarson,
L Törkvist, AS Eriksson, HE Harris, E Sundberg and M D'Amato
Genes and Immunity (2016), 1–4
"Reproduced with permission from Springer Nature"

III. Presence and reactivities of antibodies directed to citrullinated peptides in a
Swedish JIA cohort
RAYA SALEH, Erik Sundberg, Monika Hansson, Linda Mathsson-Alm,
Karl Skriner, Guy Serre, Karin Lundberg, Leonid Padyukov, Helena
Erlandsson Harris
Manuscript

IV. Juvenile idiopathic arthritis patients have a distinct cartilage and bone
biomarker profile that differs from healthy and knee-injured children
André Struglics*, RAYA SALEH*, Erik Sundberg, Mia Olsson, Helena
Erlandsson Harris, Cecilia Aulin
Clin Exp Rheumatol. 2020 Mar-Apr;38(2):355-365
*These authors share first authorship

CONTENTS

LIST OF ABBREVIATIONS

ACPA	Anti-citrullinated peptide/protein antibody
AMPK	Adenosine monophosphate-activated protein kinase
ANA	Antinuclear antibodies
AOSD	Adult-onset Still's disease
APC	Antigen presenting cell
ARGS	Aggrecanase-generated neoepitope of aggrecan
ATP	Adenosine triphosphate
AU	Arbitrary unit
AUC	Area under the curve
CARRA	Childhood Arthritis and Rheumatology Research Association
C2C	C2C neoepitope of collagen type II
CCL	C-C motif chemokine ligand
CXCL	C-X-C motif chemokine ligand
CCP2	Cyclic citrullinated peptide, second generation
CCR	C-C chemokine receptor type
CD	Crohn's disease
CEP1	C-terminal conserved extension peptide 1
COMP	Cartilage oligomeric matrix protein
COX	Cyclooxygenase
CTLA4	Cytotoxic T-lymphocyte-associated protein 4
CTX	C-terminal telopeptide of type I collagen
CXCR	C-X-C motif chemokine receptor
DMARDs	Disease-modifying antirheumatic drugs
DAMPs	Danger-associated molecular patterns
DNA	Deoxyribonucleic acid
IG	Immunoglobulins
ECM	Extracellular matrix
ELISA	Enzyme-linked immunosorbent assay.
ERA	Enthesitis-related arthritis
EULAR	European League Against Rheumatism
GAG	Glycosaminoglycan
GWAS	Genome wide association study
HLA	Human leukocyte antigen

hnRNP	Heterogeneous nuclear ribonuclear protein
IBD	Inflammatory bowel disease
IFN	Interferon
IL	Interleukin
ILAR	International League of Associations for Rheumatology
IL2RA	Interleukin-2 receptor alpha
JIA	Juvenile Idiopathic Arthritis
LACC1	Laccase domain-containing 1
MAS	Macrophage activation syndrome
MHC	Major histocompatibility complex
MIF	Macrophage migration inhibitory factor
MMP	Matrix metalloproteinases
MRI	Magnetic resonance imaging
MTX	Methotrexate
NET	Neutrophil extracellular traps
NFκB	Nuclear factor-kappa B
NOD2	Nucleotide-binding oligomerization domain-containing protein 2
NSAID	Non-steroidal anti-inflammatory drugs
NTX	N-telopeptide of type I collagen
OA	Osteoarthritis
PAD	Peptidylarginine deiminase
PRR	Pattern recognition receptor
PIN	Personal identification number
PRINTO	Pediatric Rheumatology International Trials Organization
PTPN2	Protein tyrosine phosphatase non-receptor type 2
PTPN22	Protein tyrosine phosphatase non-receptor type 22
RA	Rheumatoid arthritis
RANK	Receptor activator of nuclear factor kappa-B
RANKL	Receptor activator of nuclear factor kappa-B ligand
RF	Rheumatoid factor
ROS	Reactive oxygen species
SF	Synovial fluid
SLE	Systemic lupus erythematosus
SNP	Single nucleotide polymorphism
STAT4	Signal transducer and activator of transcription 4

TCR	T-cell receptor
Th	T helper
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TNFAIP3	TNF-α induced protein 3
TRAP	Tartrate-resistant acid phosphatase
UC	Ulcerative colitis
VEGF	Vascular endothelial growth factor

1 INTRODUCTION

1.1 JUVENILE IDIOPATHIC ARTHRITIS

1.1.1 Diagnosis and prevalence of JIA

Juvenile idiopathic arthritis (JIA) is the most common chronic rheumatic disease in children with swelling, pain, stiffness, and restricted joint movement in at least one of the joints. It is characterized by both articular and extra-articular manifestations. JIA is an inflammatory progressive disease with disabilities such as joint pain and destruction, decreased range of joint mobility, growth, and physical functions impairment in children [1, 2]. The prevalence of JIA fluctuates due to variances in diagnostic criteria, low disease occurrence, and limited study sizes. The reported incidence of this condition ranges from 11 to 22 cases per 100,000 children per year in the Nordic countries and from 1.6 to 23 cases per 100,000 children per year worldwide, with a predominance among females [3-5]. According to the revised international league of associations for rheumatology (ILAR) classification criteria, it is divided into seven subtypes and each subtype has its own exclusion criteria. Common in all of them is that arthritis has begun before the age of 16, persisted for more than 6 weeks and are of unknown origin [6]. The features of the seven subtypes are summarized in table 1[6, 7].

Table 1, ILAR classification of juvenile idiopathic arthritis (2001).

Oligoarticular	Engagement of one to four joints within the initial six months of the condition, with additional joint involvement developing gradually, subsequently categorized as either persistent or extended.
Polyarticular, RF-negative	Engagement of five or more joints within the initial six months, with further specification based on the absence of rheumatoid factor.
Polyarticular, RF-positive	Engagement of five or more joints within the initial six months, with further specification based on the presence of rheumatoid factor.
Enthesitis-related	Characterized by the occurrence of arthritis and enthesitis (inflammation at the junctions of ligaments and tendons). This condition is linked to sacroiliitis, resulting in frequent lower back pain, HLA-B27 positivity, arthritis associated with inflammatory bowel disease.
Psoriatic	Arthritis accompanied by psoriasis, often accompanied by dactylitis, alterations in nail health, or a family history of psoriasis.
Systemic	Arthritis accompanied by symptoms like fever, skin rash, swollen lymph nodes, enlarged liver and spleen, and serous membrane inflammation. It is believed to be a systemic auto-inflammatory condition with a unique underlying mechanism when compared to other types of JIA [8].
Undifferentiated	A persistent form of idiopathic arthritis that doesn't align exclusively with a single category or corresponds to multiple categories mentioned above.

The most common subtype of JIA (around 40-50% of all patients) is oligoarticular JIA with

a maximum of four joints being affected during the first 6 months of disease. If additional

numbers of joints, get affected at a later stage of disease it is considered as extended otherwise

it is considered as persistent oligoarticular JIA. The oligoarticular subtype has the highest

association with presence of anti-nuclear antibodies (ANA) and together with early disease

onset is the highest risk for developing uveitis [9]. Polyarticular JIA, the second most

common subtype (around 30% of all patients) is defined when more than four joints being

affected during the first 6 months of the disease. It is further subdivided depending on the

presence or absence of Rheumatoid factor (RF) [10]. The polyarticular rheumatoid factor

negative JIA occurs in childhood or adolescence and is characterized by symmetrical arthritis

including smaller joints in hands and feet and it accounts for around 20-25% of JIA cases. It

may be ANA positive or negative with characteristic increase of the risk of uveitis.

Polyarticular RF positive form of JIA is the most similar form to adult Rheumatoid Arthritis

(RA) [11] . Disease onset is usually at older spectrum of childhood with 90% being girls and it consists of only 5-10% of all JIA patients. Anti-citrullinated protein/peptide antibodies (ACPA) have been mainly found in this group and together with RF associate with joint damage [12]. Psoriatic arthritis, presents with arthritis of larger and/or smaller joints, is diagnosed in around 5-10% of JIA patients. This diagnosis requires psoriatic skin manifestation of the patient or a first-degree relative having psoriasis. Enthesitis-related arthritis develops in later childhood or adolescence and is applied to patients with inflammation of the enthesis (the connective tissue between tendon or ligament and bone) and/or the axial spine as well as peripheral joint disease. This subtype corresponds to around 5-10% of JIA patients. Systemic JIA (sJIA) accounts for around 5-10% of the JIA patients and is characterized by arthritis and systemic inflammation with spiking fever, salmon pink skin rash, lymphadenopathy, and hepatosplenomegaly. SJIA displays distinctive clinical characteristics and therapeutic responses reminiscent of auto-inflammatory diseases. Nevertheless, approximately 50% of children with sJIA experience the development of persistent, destructive arthritis that mirrors other subtypes of JIA [13-15]. sJIA bears a notable resemblance to adult-onset Still's disease (AOSD). It has been postulated that they could essentially be manifestations of the same disease occurring in distinct age groups [16-18]. Some of the sJIA and AOSD patients (around 10- 40%) can also be at risk of severe pulmonary involvement and developing macrophage activation syndrome (MAS), which is a potentially life-threatening complication characterized by immune dysregulation leading to a cytokine storm including IL-18 and interferon (IFN)-γ that causes disseminated intravascular coagulation, elevated ferritin levels, hepatic involvement, and the physiological response resembling sepsis that can lead to severe dysfunction of vital organs, occasionally resulting in a fatal outcome [19-26]. About half of patients with sJIA have self-limited disease course and half of them develop a chronic persistent arthritis that might extend into adulthood [27]. Among these patients, particularly when inadequately treated, concerns such as stunted

growth, joint destruction, and enduring disability may become significant challenges. No specific biomarker has been found to distinguish at the outset of the disease, which patients will experience an extended and challenging course [28-30]. The combination of severe systemic symptoms, the need for intensive immunosuppressive treatment, and the tendency to develop persistent, damaging synovitis make systemic JIA possibly the most perilous type of arthritis in childhood [31].

The ILAR classification system determines categories based on the clinical presentations within the first 6 months of symptom onset. All JIA categories are distinct, with specific exclusion criteria for each subtype. However, some elements of this classification system have faced criticism [17, 32]. For example, the quantification of joint involvement is a significant criterion for distinguishing disease categories in the current classification system, but it exhibits limitations in terms of reliability due to differences in interpretation among observers and a lack of agreement between clinical and ultrasonic examination. Instead, it may merely reflect the severity of the disease as indicated by the extent of joint engagement. Another significant critique revolves around the classification system's inability to differentiate diseases that affect both children and adults from those that are exclusive to children. This differentiation holds crucial significance, not only for the smooth transition of patients from pediatric to adult healthcare but also for expediting the approval of new medications. Furthermore, the existing classification system fails to recognize a sizable group of children suffering from a relatively uniform disease, a situation not observed in the adult population. This distinct group of JIA patients, are mostly female, with disease onset before the age of 6 and exhibit distinct characteristics, such as asymmetric arthritis, iridocyclitis also known as anterior uveitis, and the presence of antinuclear antibodies (ANA) [18, 33, 34]. In 2019, the Pediatric Rheumatology International Trials Organization (PRINTO) Consensus revised the ILAR classification criteria and proposed a novel classification system for JIA that does not require joint count and the onset of disease begins before the age of 18 instead

of 16. Four new principal arthritis forms were identified, entitled: systemic JIA, RF-positive JIA, enthesitis/ spondylitis related JIA, and early-onset ANA-positive JIA [18].

1.1.2 Management of JIA

As of today, there is no medication that can cure JIA, but prognosis has improved over the years due to better management of disease. At present, treatment of JIA is a combination of medication, physical therapy, and psychological therapy. The goal of treatment is to control pain, prevent long-term damage, facilitate growth and preserve physical and psychological well-being of children [35]. Pharmacological treatment of JIA includes anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, and disease modifying drugs such as methotrexate and biologics. It is difficult and remains a clinical challenge to identify which children will experience a moderate self-remitting disease course and which ones will have unremitting disease and are at risk of further complications such as joint destruction and physical impairment.

Non-steroidal anti-inflammatory drugs

Since JIA is not a single disease, the treatment regimen differs between subtypes. Early in the disease course or with mild disease, non-steroidal anti-inflammatory drugs (NSAIDs) is a treatment option. NSAIDs inhibit cyclooxygenases (COX)-1 and COX-2, the enzymes that produce prostaglandins. NSAIDs do not modify the disease but reduce systemic and local inflammation, pain, and fever. However, NSAIDs may also cause gastrointestinal side effects [36, 37].

Corticosteroids

Corticosteroids are potent short-term anti-inflammatory medications used in many different inflammatory diseases and conditions. They suppress the immune system but are not capable of reducing the progression of the disease in long term. The mechanisms of action of corticosteroids involve decreased production of pro-inflammatory mediators including TNF and IL-1. Corticosteroids are administered either as local intra-articular injections or systemically. Corticosteroids should be administered with extreme caution due to their possible harmful impacts, particularly concerning bone health and the growth of children [38, 39].

Disease-modifying antirheumatic drugs (DMARDs)

DMARDs are a category of drugs defined by their use in Rheumatoid Arthritis (RA) for their ability to provide long-term effects and slow down the progression of the disease. The term is often used in contrast to NSAIDs and corticosteroids [40].

Methotrexate

There are several DMARDs used in the treatment of rheumatic diseases. Methotrexate (MTX) is often considered as the first choice of DMARDs in JIA and the second line candidate for treatment of JIA patients with persistently active arthritis because of its effectiveness and only moderate side effects. From studies that have shown that MTX can decrease the rate of disease progression it has been indicated that MTX may have a disease-modifying effect. MTX was first developed as a cancer treatment because of the cell proliferation inhibitory effects caused by antagonizing folate and interfering with DNA synthesis at high doses. In low-doses, MTX also acts as an immunomodulatory and anti-inflammatory drug. The exact mechanism of MTX action in rheumatic diseases remains

unknown, but it is believed to affect the cellular production of a range of cytokines and thus suppressing cell mediated immunity [41, 42].

Biologics

During last decades, better understanding of the immune system and the pathways involved in the pathogenesis of diseases has made it possible to develop targeted therapies against key pathogenic mediators. The greatest improvement in the treatment of RA and then JIA patients has been achieved by the introduction of highly specific biological treatments. For example, targeting of key cytokines including tumor necrosis factor alpha (TNF-α) (in non-systemic JIA), IL-1 and IL-6 (in systemic JIA) as well as signaling molecules involved in regulation of T-cell and B-cell responses have been successful in decreasing of disease activity [43-46].

JAK-STAT inhibitors

JAK-STAT inhibitors represent a novel therapeutic approach involving small molecules that specifically target the JAK (Janus kinase)-STAT (signal transducer and activator of transcription) signaling pathway downstream of cytokines and growth factor receptors. These compounds act by hindering the phosphorylation of JAK enzymes or STATs, a critical step triggered by the activation of various cytokines (like type I and II interferons, IL-2, IL-6, and IL-10) as well as growth factor receptors. Consequently, this interference downregulates downstream signaling cascades altering the transcription of genes responsible for modulating key cellular processes including the regulation of cell growth, differentiation of immune cells including Th1& Th17 cells and plasmablasts, inflammatory reaction and inhibit the function of various inflammatory signaling molecules.

1.2 ETIOLOGY OF JIA

1.2.1 Environmental factors

JIA is a complex disease and the exact etiology of it is not fully understood. It is believed to result from environmental exposures in individuals with a genetic predisposition [47]. Several environmental factors have been suggested to influence JIA. Some studies suggest that breastfeeding and having siblings may have a protective effect on the development of JIA by influencing gut microbiota. Conversely, bacterial, and viral infections, antibiotic usage, and cesarean (C-section) delivery may impact the microbiome diversity for extended periods of time, potentially posing risks for the development of JIA. On the contrary, the impact of various potential factors, such as exposure to second-hand smoke, environmental pollutants (both air and occupational exposures), Risk factors occurring during the perinatal period (such as maternal age, birth weight, multiple births, congenital anomalies, season of birth, or maternal-child blood group mismatch), dietary influences, sun exposure, and vitamin D, is less well-defined. Studies from diverse populations have yielded varying and at times conflicting outcomes, ranging from no apparent effects to significantly positive associations, as well as subtle inverse associations [48-50].

1.2.2 Genetics

Evidence of a genetic component for JIA is based on twin and familial studies. Monozygotic twin concordance rate is between 25- 40%. Siblings and cousins are reported to have a 11.6 and 5.6-fold increase, respectively, in the risk of JIA compared to general population [51, 52]. Additionally, there is a higher occurrence of other autoimmune disorders, especially rheumatoid arthritis, in the family members of individuals with JIA. Moreover, there is evidence of shared genetic susceptibility loci between these two conditions [53].

Several susceptibility loci have been reported in JIA [54-57]. Similar to various other autoimmune disorders, the most significant identified genetic susceptibility factors for JIA are linked to human leukocyte antigen (HLA) locus at chromosome 6. The HLA locus includes genes encoding class I (HLA A, B, and C), and class II (HLA-DR, DP, and DQ) molecules [58]. The HLA class I and class II haplotypes that are associated with the risk of JIA have both similarities and differences with those connected to rheumatoid arthritis and other autoimmune diseases as well as among different subtypes of JIA [33, 54, 59, 60]. It was estimated that approximately 13% of JIA risk can be attributed to the HLA locus, while the top 27 non-HLA loci identified by genome-wide association studies (GWAS) and found mainly in oligoarticular JIA and RF-negative polyarticular JIA account for about 6% of the risk. This suggests that there are many more risk factors associated to JIA that need to be discovered [54, 55]. GWAS compares the frequencies of common genetic variants in unrelated cases and controls. This method contributed to identification of thousands of loci associated with various diseases and traits by examining hundreds of thousands to a million single nucleotide polymorphisms (SNPs) that represent common genetic variations in the human genome. A successful GWAS reveals specific genetic variants within a locus, indicated by the associated tag SNP, that may play a role in the observed disease or trait association [61, 62]. The impact of each genetic variant usually is relatively modest, with odds ratios ranging from 6 for the HLA region down to 1.1–1.6 for other genetic loci. It's crucial to understand that these odds ratios primarily reflect the impact of common genetic variations (minor allele frequency >1%) identified through genome-wide association studies. Typically, common variants have relatively modest functional consequences due to evolutionary pressures, resulting in correspondingly minimal effects on disease risk. However, these values should not be misconstrued as indicative of the significance of specific genes and often is not easy to interpret in context of specific phenotype rather than a

biomarker. Additionally, genetic associations offer valuable insights when examined at the population level [33].

The HLA gene complex located at chromosome 6p21.3, has the strongest genetic association to JIA with particular importance of *HLA-DRB* and *HLA-DQA/B* genes. Various HLA associations have been found to different JIA subsets. It has been reported that HLA-*DRB1*08, DQA1*04* and *DQB1*04* alleles confer risk to oligoarticular JIA and RF negative polyarticular JIA. However, *DQA1*04* and *DQB1*04* are in high linkage disequilibrium (LD) with the *DRB1*08* allele and it is not clear which allele is an independent contributor to this association. Oligoarticular JIA and RF negative polyarticular JIA exhibit significant genetic similarities. On the other hand, oligoarticular JIA has shown associations with HLA-*DRB1*01, HLA-DRB1*11, HLA-DRB1*13*, *HLA-DPB1*02*, and *HLA-DQB1*04* alleles. Meanwhile, *HLA-DRB1*04* and *HLA-DRB1*07* appear to have a protective role in this context. Furthermore, RF negative polyarticular JIA has also demonstrated an association with *HLA-DPB1*03* and *HLA-DRB1*15* allele [58, 63, 64].

*DRB1*04* allele which is a risk allele for RF positive RA is associated with susceptibility to RF positive polyarticular JIA [63, 65]. RA associated shared epitope coding *HLA-DRB1* alleles have a significantly higher frequency in RF positive polyarticular JIA compared to controls. The *HLA-DRB1* alleles known to carry the shared epitope sequence (five amino acid residues at position 70-74 in the third hypervariable region of the HLA-DRB1 chain), include: *HLA-DRB1*01:01, *01:02, *01:05, *04:01, *04:04, *04:05, *04:08, *04:09, *04:10, *04:13, *10:01, *14:02, *14:06* [54, 66].

In the case of enthesitis JIA, the primary haplotype implicated is *HLA-B*27*, which is also associated with ankylosing spondylitis in adults. In PsA, the principal HLA alleles shown

involved include *HLA-DRB1*08*, *HLA-DQA1*0401*, *HLA-DQB1*0402*, and *HLA-B*27* [65].

sJIA has shown a unique genetic association compared to other JIA subtypes [8]. *HLA-DRB1*11-DQA1*05-DQB1*03* haplotype has been associated with sJIA in several different populations with *DRB1*11* allele being recognized as the major hit [33, 64, 67].

Several non-HLA genes have been associated to JIA as well. A missense variation in protein tyrosine phosphatase N22 (*PTPN22*) gene is the best-validated non-HLA autoimmune susceptibility risk factor (odds ratio of around 1.5) to JIA [68]. It has been a well-established susceptibility locus to other autoimmune diseases such as type 1 diabetes, RA and SLE, multiple sclerosis and Crohn's disease as well. The *PTPN22* gene located on chromosome 1p13.2, encodes for a protein tyrosine phosphatase PTPN22, whose role is to negatively regulate T cell activation [68]. An intronic SNP in the signal transducer and activator of transcription 4 (*STAT4*) gene has been associated to JIA as well as RA and SLE. *STAT4* is involved in IL-12 signaling and important in differentiation of Th cells [57, 69]. Association with JIA was reported for the polymorphisms within genes encoding cytokines TNF, IL-10, IL-6, macrophage migration inhibitory factor (MIF), the interleukin 2 receptor alpha (IL2RA) [70], IL-2 [70], TNF-α induced protein 3 (TNFAIP3) [71], CC chemokine receptor 5 (CCR5) [72]. *PTPN2*, a gene located on chromosome 18p11.21, encodes the tyrosine phosphatase PTPN2 and is similar to PTPN22 in its function of regulating T and B lymphocytes. This gene is linked to susceptibility not only in JIA but also in other autoimmune conditions like type 1 diabetes, RA, and Crohn's disease [57]. The above non-HLA associations with JIA have not been found in sJIA supporting the evidence for being a distinct disease from other JIA categories [65, 68]. Overall, these genetic associations prove that established JIA risk loci play significant roles in regulating and functioning within the immune system [57].

JIA is a complex polygenic disease, because of intricate interplay between a predisposed genetic makeup, encompassing both HLA and non-HLA gene variants, and multiple environmental elements, demonstrated by the lack of complete concordance between siblings and even between identical twins [73, 74]. In rare cases, the development of JIA has been related to genetic defects in a single gene with development of Mendelian type of disease [75]. Utilizing next-generation sequencing to study familial cases of JIA, researchers identified mutations in the *LACC1* (*laccase domain-containing 1*) gene, making it the first example of autosomal recessive juvenile febrile polyarthritis with similarities to systemic JIA (sJIA), hinting at abnormal activation of innate immunity [76]. Interestingly, *LACC1* rare genetic mutations have also been found in other forms of JIA as well [77]. Furthermore, genome-wide association studies (GWAS) have uncovered *LACC1* polymorphisms associated with immune and bacterial diseases, such as inflammatory bowel diseases (IBD) and leprosy [77-81].

Overall, genetics of JIA is pending further investigation and integration of this knowledge with functional dysregulation that happens in immune system during development of JIA.

1.3 HISTOPATHOLOGICAL FEATURES OF JIA

Inflammatory joint destruction is the hallmark of JIA. Within the synovial joint, the synovial membrane undergoes thickening as a result of uncontrolled synoviocyte proliferation and the infiltration of various immune cells. These immune cells encompass T cells, B cells, natural killer cells, neutrophils, macrophages, dendritic cells, and plasma cells, which infiltrate the sub-lining layer of the synovium. This leads to hyperplasia and hypertrophy of the synovium, resulting in reduced oxygen levels within the joint. Consequently, this environment promotes the production of pro-angiogenic factors, triggering abnormal blood vessel formation [82,

83]. Elevated levels of vascular endothelial growth factor (VEGF), along with its soluble receptors and osteopontin, have been associated with synovial angiogenesis in JIA patients. The formation of new blood vessels within the synovium enhances blood supply and facilitates the influx of pro-inflammatory cells into the joint, leading to the development of pathological synovial tissue known as pannus [84]. In the subintimal region of the joint, granulocytes, macrophages, plasma cells, and lymphocytes accumulate and release pro-inflammatory molecules like tumor necrosis factor (TNF) and interleukin-1β (IL-1β). These substances upregulate pannus-synoviocyte production of catabolic enzymes, including matrix metalloproteinases (MMPs), aggrecanases, and cathepsins. These enzymes degrade the extracellular matrix of the articular cartilage, causing loss of function and destructional damage [85, 86]. Furthermore, pro-inflammatory cytokines stimulate receptor activator of NF-kB (RANK)-expressing osteoclasts, resulting in bone erosion. In advanced stages, this damage to cartilage and bone can lead to ankylosis, causing a loss of mobility in the affected joints. Considering that JIA primarily affects children, it can disrupt normal skeletal growth, potentially leading to long-term issues [82, 83, 87, 88]. Overall, histological changes in JIA reflect continuous inflammation and immune reactions locally in joints and surrounded tissues.

1.4 PATHOGENIC MECHANISMS IN JIA

The hallmark of JIA, similar to rheumatoid arthritis (RA) in adults, is an inflamed and thickened joint synovium that contains a dense inflammatory infiltrate. Since JIA is not a single disease but a spectrum of conditions with varying degrees of overlap, the pathogenesis differs for each subtype and may also share common mechanisms within this spectrum. Dividing JIA patients into different subtypes is primarily based on clinical manifestations, and it reveals the ambiguity surrounding the underlying biological factors contributing to the

heterogeneity of the disease. JIA is thought to be caused by dysregulation of immune system as a result of both genetic and environmental factors [47]. Even though the various JIA subtypes are likely to have distinct underlying pathophysiological mechanisms, most forms of JIA seem to originate from the failure of immunologic self-tolerance. The levels of immune cells like lymphocytes, monocytes, and neutrophils in peripheral blood are differentially increased in different subtypes [89]. The adaptive immune response plays an essential role in pathogenesis of non-systemic subtypes of JIA. Autoreactive T and/or B cell activation has been indicated in oligo- and polyarthritis patients [86, 90]. Many genetic studies have indicated subtype specific associations with HLA alleles that supports involvement of adaptive immunity, although it is not clear what triggers this self-reaction. In fact, the strongest genetic association is major histocompatibility complex (MHC) class II alleles, that codes for the B chain of MHC class II molecules. As the role of Class II HLA molecules is to present peptide antigens on the surface of antigen-presenting cells (APCs) to be recognized by the T cell receptors (TCR) on the CD4+ cells, this strongly suggests activation of adaptive immunity specifically T cells [91]. Of the CD4+ T helper (Th) cells, both Th1 and Th17 populations have been observed to be enriched in the joints compared to the peripheral blood. Th1 cells produce Interferon gamma (IFNγ) and result in a mononuclear infiltrate in the joints. Th17 cells are also abundant in the joints of JIA patients and produce high levels of their signature cytokine, IL-17 as well as other cytokines such as IL-6, TNF, IL-21. *In vitro* studies have shown that IL-17 induces production of cytokines such as IL-6, IL-8 and matrix metalloproteinases (MMPs), that operates to break down tissue as part of an inflammatory reaction, TNF, chemokines, and chemoattractants for neutrophils from fibroblasts and macrophages. One significant consequence of these effects is the activation and recruitment of neutrophils to the site of inflammation [92-94]. Inflamed joints in children with oligoarticular, polyarticular, and systemic JIA (sJIA) exhibit an unusual ratio of Th17 to regulatory T cell subsets in their synovial fluid. Additionally, the number of Th17 cells

positively correlates with the severity of arthritis [93]. Although each Th1 and Th17 cell response can cause an autoimmune disease independently of one another, there is evidence that they counter-regulate and supplement each other as well. It has been shown that lack of one of the specific cytokines produced by each cell type *in vivo* promote a response dominated by the other and targeting only one of these may exacerbate the other reaction [95, 96]. On the other hand, binding of TCR to HLA class II receptor activates signaling pathways through co-receptors in APCs that in turn regulates their function and survival such as regulation of Toll-like receptor signaling in monocytes and dendritic cells and inducing expression of proinflammatory cytokines [97-99]. There is an increased level of monocyte derived inflammatory cytokines of innate immune system in JIA. Monocytes in the SF of JIA are highly activated and secrete a variety of cytokine such as IL-6, IL-1β, TNF and chemokines, such as CCL5, CXCL10 and IL-8 [100]. It has also been shown that neutrophils in SF from polyarticular JIA have a differential expression of over 700 genes compared to healthy controls. These differences remained after clinical improvements due to medical treatments, suggesting for an involvement of neutrophils in the pathogenesis of polyarticular JIA [101].

Another way CD4+ T cells play a significant role in the pathogenesis of JIA is by facilitating the activation of autoreactive B cells. In essence, recognition of self-antigens by specific T and B cells enables CD4+ T cells to promote the affinity maturation within B cell clones by inducing secondary V(D)J-recombination of the immunoglobulin genes and isotype switching in the immunoglobulin heavy chain of memory B cells and plasmablasts secreting IgG within the synovial fluid of affected joints [102-104].

Role of B cells as precursors of autoantibody producing plasma cells in pathogenesis of JIA is not well understood yet. Autoantibodies reacting with different tissue autoantigens such as RF, ACPA and ANA can be detected in sera of JIA patients with some subtype of disease. However, their direct pathogenic role remains unknown. It is important to consider the role

of B cells in the pathogenesis not only as autoantibody-producing cells but also as antigen-presenting cells (APCs) to T-cells in synovium and thereby amplifying pathogenic T cell responses [105].

The primary immunopathological feature that sets sJIA apart from other JIA subtypes is uncontrolled activation of innate immunity in the center of pathogenesis which differs from other JIA subtypes where adaptive immunity plays a more significant role. There is an excessive expression of monocyte-derived proinflammatory cytokines, including IL-1, IL-6 and ́IL-18 which classifies it more as an auto-inflammatory disease rather than autoimmune in other JIA subtypes. sJIA also differs in distinct clinical characteristics and treatment responses, resembling those seen in autoinflammatory diseases especially adult-onset Still's disease (AOSD). Successful treatment of JIA with IL-1 and IL-6 inhibitors such as anakinra (recombinant form of the natural IL-1 receptor antagonist), and tocilizumab (a humanized anti-IL-6 receptor monoclonal antibody) proves the involvement of innate immune cytokines in the pathogenesis of JIA- particularly in sJIA [13, 106].

Elevated levels of alarmin S100 proteins secreted from monocytes and granulocytes have been observed in autoinflammatory disease including sJIA. Once these cytokines and proteins are released into the extracellular environment, they can initiate proinflammatory responses in human monocytes through a toll-like receptor 4 (TLR4)-dependent mechanism. Moreover, they can also stimulate the proinflammatory activation of vascular endothelial cells and up-regulation of adhesion molecules on endothelial cells. This, in turn, facilitates the adherence of neutrophils and other leukocytes, followed by their migration from the bloodstream into the surrounding tissue, ultimately promoting localized inflammation [107-111].

Although the available evidence regarding autoimmunity in systemic JIA is limited, there is some data that suggest a potential involvement of T lymphocytes in its underlying mechanisms, especially in the subtype of sJIA characterized by a predominant arthritis

course. In fact, while the excessive activation of the innate immune system through IL-1 and IL-6 in an antigen-independent manner initiates a cascade involving endothelial cells, leukocytes, and various tissue cell types, leading to daily fever and systemic inflammation during the initial stages of sJIA, these cytokines also could incite T-cell activation, leading to the development of chronic arthritis [31, 112].

It has been demonstrated that IL-1 and IL-6 play a role in promoting the differentiation of undifferentiated T cells into the Th1 and Th17 phenotype. Furthermore, they induce the expansion and activation of proinflammatory T cells, leading to the production of IL-17 and IFN-γ all while diminishing the susceptibility of effector T cells (Teff) to suppression by regulatory T cells (Treg) [113]. It has also been noted that peripheral blood of children with sJIA exhibit a higher ratio of proinflammatory IFN-γ producing Th1 and IL-17 producing Th17 cells compared to healthy controls [114].

At the genetic level, despite the many genetic distinctions between sJIA and other JIA forms, the most robust genetic association with sJIA remains the MHC class II allele *DRB1*11*. These findings reinforce the idea that autoreactive CD4+ T cells play a pivotal role in the pathogenesis of all JIA subtypes [8, 67]. Moreover, abatacept (CTLA4-Ig) which hinders T cell activation by inhibiting costimulatory signals via CD80 and CD86, receptor of antigen presenting cells, has been an effective treatment for children with chronic articular sJIA [115, 116]. Overall, the pathogenetic mechanisms of JIA remain mostly unknown, and our knowledge about the disease, including the distinctions between JIA subtypes, is pending further investigation.

Extensive recent studies in both mice and humans have shown multiple roles of LACC1 in excessive innate immune activation. LACC1, also known as C13ORF31 or FAMIN, is a 47-kilodalton protein primarily expressed in macrophages [117]. These roles encompass lipid

metabolism, host defense against bacterial infections, as well as vital contributions to the Pattern Recognition Receptor (PRR)-induced mitochondrial ROS (mtROS) and NFκB pathway activation, inflammasome function, cytokine secretion, and intracellular bacterial clearance [118]. Interestingly, a recurring theme in these investigations is the reduced functionality of macrophages, especially in their ability to produce cytokines and reactive oxygen species when LACC1 is deficient. This diminished performance reflects an altered interaction between the host's immune system and microorganisms [119].

Recent proteomic and biochemical investigations have revealed a new dimension of LACC1's function, emphasizing its role in the accumulation of lipid droplets within macrophages. These lipid droplets serve as a source of energy for mitochondrial respiration through the oxidation of fatty acids, which is crucial for the energy needs of the cell, including immune responses thereby regulating autophagy and phagocytosis within macrophages. It is suggested that LACC1 interacts with AMPK (adenosine monophosphate-activated protein kinase), a key enzyme regulator of cellular energy homeostasis and an initiator of autophagy. Autophagy is a process through which cells degrade and recycle their components, including damaged or excess organelles and components including proteins and lipids [120, 121]. AMPK can regulate autophagy in response to changes in energy levels within the cell. It is activated in response to low cellular energy levels, such as when ATP (adenosine triphosphate) levels are low or when there is an energy deficit. When AMPK is activated, it promotes catabolic processes that generate ATP and inhibit anabolic processes, including the synthesis of lipids, which are stored in lipid droplets and promoting their breakdown for energy production. This helps cells cope with energy deficits and maintain energy balance. Reduced AMPK activity leads to decreased autophagy [119, 122]. In LACC1-deficient macrophages, whether derived from patients or generated through gene knockdown, there is a decreased capacity to uptake apoptotic bodies and phagocytose bacteria compared to the control group. These findings underscore LACC1's critical role in modulating essential

macrophage processes like phagocytosis through autophagy regulation. These results emphasize the influence of the extracellular matrix and tissue microenvironment on LACC1-associated phenotypes. It is reasonable to speculate that the metabolic composition of the joint microenvironment can drive a pro-inflammatory phenotype in LACC1-deficient macrophages. Alternatively, autophagy in macrophages, which relies on LACC1, might facilitate the removal of specific pro-inflammatory lipids found in the joints [119]. Studies involving LACC1-deficient mice have unveiled that they exhibit increased inflammatory responses to immune stimuli. This exaggerated response may be due to the abnormal function of energy-deprived macrophages, which produce higher levels of TNF and IL-17, subsequently promoting Th17 cell development and intensifying experimental arthritis and colitis under inflammatory conditions. Nevertheless, spontaneous inflammatory phenotypes were not detected in these mice [117]. In essence, LACC1's involvement in the accumulation of lipid droplets within macrophages, its impact on autophagy, and its role in regulating vital immune functions present it as a promising player in the pathogenesis of various inflammatory diseases, including arthritis. However, a precise understanding of LACC1's role in pathophysiology requires further investigation and clarification.

Crohn's disease is a specific form of inflammatory bowel disease (IBD) characterized by inflammation that can extend through the entire bowel wall. It is believed to be caused by disrupted interactions between the host and gut microbes, leading to abnormal cytokine production [123]. These interactions are controlled by host pattern recognition receptors (PRRs). Dysfunctions in PRR signaling, whether caused by reduced or increased activity, can contribute to intestinal inflammation. Maintaining a delicate balance in regulating PRR-mediated responses is crucial for gut health [124]. The significance of host-microbial interactions is underscored by genetic factors associated with Crohn's disease (CD). Loss-of-function mutations in NOD2, a PRR responsible for detecting bacterial peptidoglycan, are linked to CD. Additionally, other genetic pathways that affect microbial clearance

mechanisms, such as autophagy and the generation of reactive oxygen species, are implicated in CD [125, 126].

The current evidence suggests that JIA patients face an elevated risk of immune-related gastrointestinal conditions, specifically Crohn's disease and ulcerative colitis. In fact, when looking at the entire spectrum of JIA patients, the incidence of IBD is notably higher, with rates ranging from 20 to over 40 times those observed in the broader pediatric population [127-129]. Although IBD has been reported in different subtypes of JIA, it is most frequently reported in ERA subtype which are more often male and *HLA-B27* positive than JIA patients without IBD [34, 128, 130, 131]. ERA resembles adult spondyloarthropathy, a rheumatic condition that is recognized for its connection to the onset of IBD [132, 133]. JIA patients who have systemic JIA, psoriatic arthritis or extended oligoarthritis have been found to be at a higher risk of developing IBD as well [128, 129].

1.5 AUTOANTIBODIES IN JIA

1.5.1 Antinuclear antibodies (ANA)

Antinuclear antibodies (ANA) are autoantibodies that have been studied for over six decades and have high sensitivity (90%) for diagnosing SLE in both adults and children, but they are also detected in patients with various autoimmune conditions including JIA, mixed connective tissue disorder, juvenile dermatomyositis, Sjogren's syndrome, scleroderma, autoimmune hepatitis, ulcerative colitis, and autoimmune thyroiditis [7, 134-137].

While there are different tests available for identifying ANA, the most frequently employed screening methods include indirect immunofluorescence and enzyme-linked immunosorbent assay (ELISA). The former employs a range of tissue substrates, while the latter utilizes a blend of antigens (either purified or recombinant), offering notably high specificity and

sensitivity, coated onto polystyrene plates [138]. While the presence of ANA is more common in children with JIA than in healthy children, it is important to note that the ANA test does not significantly increase the risk of JIA and is not currently used as diagnostic tool in JIA. However, it holds significant prognostic value regarding the risk of uveitis. The prevalence of a positive ANA among all subtypes of JIA is generally less than 50% [139]. Furthermore, false-positive results and transient positivity of the ANA, often due to infections, are not uncommon [140]. The prevalence of ANA positivity varies among different JIA subtypes. Up to 70% of patients with oligoarticular JIA, particularly young females, exhibit higher ANA positivity rates. Likewise, in the case of psoriatic JIA patients, ANA positivity is linked to the onset of the disease at an early age and exhibits a higher prevalence among females. However, the presence of ANA is less frequent in undifferentiated JIA and systemic JIA patients. It's worth noting that a recent study revealed an increasing trend in ANA and rheumatoid factor titers among patients with systemic JIA over time [34, 141, 142].

In terms of JIA prognosis, the literature offers mixed evidence concerning the relationship between ANA status and arthritis outcomes. Large-scale studies have not consistently shown significant differences related to ANA positivity and the number of active joints at follow-up, remission rates on or off medication, or the prediction of relapse [139, 143]. However, a study that used a protein-based microarray to investigate sera of JIA patients with oligoarticular JIA concluded that specific autoantibodies targeting nuclear antigens such as histone and chromatin may serve as better biomarkers for prognosis and predicting active JIA during the five-month follow-up, compared to the standard ANA test [144].

Etiological hypothesis of ANA

Besides ANA relevance as a biomarker for specific JIA phenotypes within clinical definition of the disease, there is a lack of knowledge regarding role of ANA in JIA pathogenicity.

However, several studies that have investigated role of ANA in other autoimmune diseases, have provided evidence for their involvement in pathogenesis. First the presence of these antibodies several years before the onset of the disease without any clinical symptoms has been shown in scleroderma and SLE. Second, higher titers of ANA have been observed in patients compared to controls. The levels vary over time in patients, and it correlated with disease activity in Scleroderma patients and B-cell depletion with rituximab which is an anti-CD20 medication was beneficial in these patients [145]. Furthermore, passive transfer of ANA has led to lupus-like symptoms and exacerbation of autoimmune features in the disease prone mice leading to more severe symptoms and organ involvement [146]. Skin and lung fibrosis have been observed in mice models of sclerosis as well [147].

ANA, primarily of the IgG class, specifically target nuclear antigens that are typically 'hidden' within cells. However, under certain conditions such as abnormal apoptosis and necrosis, these antigens are exposed to outside of the cell. In such cases, dying cells may release their nuclear antigens (neoantigens) into the extracellular environment. In genetically predisposed individuals and under the influence of environmental factors, impaired clearance of these released antigens can lead to their accumulation in various tissues. Over time, this accumulation of neoantigens may disrupt immune tolerance mechanisms, potentially resulting in a loss of self-tolerance [148, 149]. The mechanism behind the generation of ANA is rooted in the immune response triggered by post-translational modifications of self-proteins and release of neoantigens outside of the cells. Additionally, molecular mimicry occurs, where cross-reactive antibodies recognize both microbial and human proteins. This can lead to the production of antibodies directed against a particular antigen. Subsequently, this initial immune response may cascade into the production of antibodies against other structurally similar antigens, a phenomenon known as epitope spreading. [150-154]. Several ANA subtypes exist, each targeting distinct cellular components within the nucleus,

including DNA, histones, RNA, and nucleic acid-protein complexes [155-157]. The pathogenic mechanism suggested for ANA include the immune complex formation with nuclear autoantigens released from apoptotic bodies which activate complement system and interact with different Toll-like receptors on fibroblasts and endothelial cells and depending on the specific ANA subtype induce inflammation and secretion of extracellular matrix and proinflammatory cytokines such as IL-8 and IL-6 [146, 147].

1.5.2 Antibodies against citrullinated protein/ peptide (ACPA)

Antibodies against citrullinated protein/ peptide (ACPAs), are a group of antibodies clinically assessed by a commercial ELISA based on reactivity to a library of synthetic cyclic citrullinated peptides (second generation anti-CCP ELISA, CCP2). Detection of anti-CCP antibodies have been included in the diagnostic criteria for RA as they have a high specificity (89%–100%) and sensitivity (65%–80%) for RA. Despite being a good diagnostic tool, CCPs have been designed to identify as many antibody reactivities against different citrullinated autoantigens as possible and may not translate to real peptides *in vivo*. Investigations are ongoing to find the real ACPA targets that will help in better understanding of the pathogenesis and aid in finding more specific therapies [158, 159]. ACPA predict progression to RA in patients with undifferentiated arthritis [160]. ACPA is associated with worse prognosis, poor outcome and they have important prognostic value for assessing radiological damage in RA. They have also been associated with pain, which appears to be independent of inflammation [161-168]. ACPA can be detected in blood of RA patients several years before clinical symptoms occur and the antibody level and number of reactivities increases as it gets closer to the diagnosis [169]. This may suggest that they have a role in disease pathogenesis [169-171]. An increased level of citrullination has been observed in RA. More specifically, several citrullinated proteins have been detected in synovial tissue in rheumatic joints and circulating antibodies against them have been detected in patients not only in

blood, but also in synovial tissue. Anti-citrullinated type II collagen, fibrinogen, filaggrin, α-enolase and vimentin antibodies are the best described ACPA fine specificities, although these reactivities are known to be highly overlapping [95, 172-174].

The role of anti-CCP antibodies in children with JIA has been less studied [175]. In JIA, the presence of ACPA has also been detected in ranges between 2 to 24% of patients [8, 9] [12, 176-180], depending on the specific JIA subtype in the studied cohort, which is much less prevalent and with lower levels than in RA [181, 182]. According to a meta-analysis, it was reported that anti-CCP antibodies have a heterogenous sensitivity and specificity depending on the studied population and it's subtypes and with the higher sensitivity of detection in RF-positive polyarthritis JIA (48%) than in the other subtypes (17%) and a high specificity ranging from 97% to 100% [183]. In a number of studies, ACPA has been associated to an erosive joint disease or radiological damage and severity of disease course in JIA, more specifically in polyarticular RF-positive subset [12, 179, 184-187]. The detection of ACPA in polyarticular RF positive JIA has been consistently associated with an increased likelihood of experiencing a more aggressive and erosive disease [180, 187-189]. This pattern is akin to that observed in adult RA patients who are ACPA-positive, as they tend to exhibit a more severe disease progression [190, 191]. Similar to RF, the presence of ACPA in children with polyarticular JIA often prompts pediatric rheumatologists to recommend earlier and more intensive therapeutic interventions [192]. Identification of the target proteins of citrulline modification in JIA has not been fully determined and is difficult to assess due to broad overlap between ACPA reactivities. However, the presence of anti-citrullinated vimentin, fibrinogen and type II collagen antibodies in JIA have been reported [193-198].

Citrullination and PAD enzymes

Citrulline is produced through an enzymatic post-translational modification process called

deimination (citrullination of arginine residues in peptide structure). It is catalyzed by Ca^{2+}-dependent peptidyl-arginine deiminases (PADs). Citrullination in general is involved in both homeostatic and disease conditions. In normal physiological situations, for example, citrullination occurs during histone modification, the terminal differentiation of epidermis and hair growth. It also occurs in the process of neutrophil extracellular traps (NETs) formation and was detected in individuals with a variety of diseases including cancer, psoriasis, multiple sclerosis, inflammatory bowel disease and rheumatoid arthritis [199].

Citrullination can occur intracellularly in the nucleus or cytoplasm and extracellularly. Several isoforms of PADs are known to exist, with PAD2 and PAD4 being linked to inflammatory conditions. These enzymes, mainly originate from neutrophils and demonstrate elevated expression in the synovial fluid of both mice and humans affected by inflammatory arthritis [200, 201]. This amino acid modification may change the structure of a protein and as a result of that it has immunogenic features since it changes the positive net charge of the arginine residues to neutral in citrulline residues. Additionally, substitution of imino group by oxygen with formation of carbonyl group slightly increases the molecular mass (~1 Dalton) for each modified amino acid residue. Cumulatively, these modifications may lead to the conformation change of the protein and may cause subsequent tolerance breach to self-proteins in genetically susceptible individuals [202-204].

Etiological hypothesis of ACPA

With discovery of ACPAs as specific biomarker of rheumatoid arthritis, the etiological role of these antibodies discussed repeatedly and the hypothesis of development of active joint inflammation was suggested [205]. Environmental factors such as smoking, silica or periodontitis can lead to a persistent local inflammation in the mucosal compartments of lungs and gums. This process triggers apoptosis in the cells and results in the activation and

release of PAD enzymes into the extracellular environment, which contains higher calcium concentrations. This leads to an increased level of protein citrullination and the generation of neoepitope peptides that were not originally encountered during thymic selection. These neoepitopes are, therefore, incapable of inducing central tolerance and can escape central tolerance mechanisms, potentially leading to the production of autoantibodies. This will enhance binding of modified peptides to *HLA-DRB1* SE that would not be bound in their native form and therefore presentation of them to T cells. In genetically susceptible individuals and in the presence of a second hit such as an infection or trauma, antigen-specific T and B cells (that have escaped negative selection) will be activated. B cells reactive to modified autoantigens and helped by class II-dependent T cells migrate and proliferate in the joint and subsequently produce ACPA. Epitope spreading and an increase in the antibody level occur over time prior to disease onset. ACPA cross-react with citrullinated epitopes present in the joints. They bind to citrullinated peptides on osteoclasts, activates them and promote bone erosion. They also induce production of chemokine CXCL8 that further promotes osteoclastogenesis. CXCL8 also binds to CXCR1 and CXCR2 on nociceptive nerves and thereby causes pain. It also acts as chemoattractant for neutrophils and induction of NETosis. Furthermore, ACPA form immune complexes with citrullinated peptides and mediate effector functions that contribute to synovial inflammation. They stimulate macrophages through both TLR-4 and Fc-gamma receptors and induce production of TNF-α and IL-6 by them. It has also been shown *in vivo* in mice models of inflammatory arthritis to exacerbate tissue damage [206-211]. Although there are repeated indications of involvement of ACPA in RA development, the etiological hypothesis of ACPA continue to serve as reasonable framework for multiple clinical and experimental studies, including studies of JIA.

1.5.3 Rheumatoid factor (RF)

Rheumatoid factor (RF), first was discovered more than 80 years ago, and has been found to be more common among patients with rheumatoid arthritis and the first clue that self-reactivity plays a role in RA [212, 213]. RF (predominantly IgM but can be of any isotype of immunoglobulins (IG)) specifically binds to the Fc portion of IgG molecules and form immune complexes that may participate in disease development. In adults, RF is most commonly associated with RA and is one of the serologic tests included in the current classification criteria for RA together with ACPA [214-216]. A meta-analysis indicated sensitivity and specificity values of detection of RF in RA as 69% and 85%, respectively [181]. RF can also be found in circulation in patients with other autoimmune disorders such as acute rheumatic fever, systemic lupus erythematosus (SLE), and Sjogren's syndrome. Furthermore, RF can be detected in individuals with infections like tuberculosis and Lyme disease, as well as in otherwise healthy individuals [181, 217].

While RF was discovered in some juvenile arthritis patients long ago, its overall presence in JIA is relatively low compared to RA, typically less than 5%. However, its presence in JIA is associated with a worse prognosis [218, 219]. In particular, JIA patients with RF positive polyarticular JIA are at a higher risk of experiencing a more aggressive disease course and bone erosion compared to JIA patients without RF [188, 220]. Consensus treatment protocols established by the Childhood Arthritis and Rheumatology Research Association (CARRA) identify RF and ACPA as unfavorable prognostic indicators in polyarticular JIA patients. This recognition leads most pediatric rheumatologists to consider more aggressive early therapies, such as TNF inhibitors, for these patients [192].

Notably, RFs play physiological roles in the normal immune system. For instance, IgM-RF promotes phagocytosis, the removal of antigen-antibody complexes during infection, complement fixation, and enhances B cell antigen uptake and presentation to CD4+ T cells

[221]. However, pathogenic IgM-RFs, as seen in RA, tend to have higher affinity and are more specific, indicating affinity maturation [222]. While RA often exhibits elevated levels of IgM-RF, with IgM-RF being the most common, the isotype-switched IgG and IgA classes are believed to be more directly linked to immunopathology and bone erosion [223]. The mechanisms underlying RF production are not yet fully understood but seem to involve immune complex recognition by B cell receptors within the context of toll-like receptor stimulation, along with T cell assistance [224, 225].

Overall, the detection of autoantibodies of various types serves as a valuable source of biomarkers in the diagnosis of rheumatic and autoimmune diseases. It also functions as a tool for studying disease mechanisms and for sub-classifying disease phenotypes. Recent studies have demonstrated that in the case of rheumatoid arthritis, systemic lupus erythematosus, and idiopathic inflammatory myopathies, autoantibody profiling aligns with the genetic, proteomic, and clinical features of the diseases, supporting the breakdown of disease complexity into more homogeneous subgroups [226-228].

1.6 JOINT DEGENERATION IN JIA

JIA may develop to a degenerative joint disease. The loss of articular cartilage during joint degeneration is irreversible and it causes both pain and limitation of joint function. This may lead to both immobility and disability. Current therapies are unable to reverse cartilage loss [229]. Inflammation induces expression of cytokines and enzymes that degrade cartilage matrix. Cleavage products from degraded matrix proteins may serve as biomarkers of destruction and can be found in body fluids like blood, urine and synovial fluid [230]. At present, the standard method for diagnosing the destruction of the joints is by clinical evaluation and radiographic techniques which detect changes in the bone structure and joint space that is caused by loss of articular cartilage. Nevertheless, radiography is less suitable for a softer tissue such as cartilage and it takes long time before changes in bone are detectable

by radiography. Thus, finding laboratory biomarkers can be helpful in detecting the early phases of joint deterioration prior to radiographic detectability and to assist response to therapy predicting future development of the disease. Furthermore, such biomarkers could be used as tools for monitoring the effects of drug treatments and could help to develop new therapies.

1.6.1 Composition and turnover of articular cartilage and bone

The bony ends of the joint are covered by articular cartilage that together with synovial fluid facilitate joint movements and protect it from mechanical shocks. Cartilage consists of a compact extracellular matrix (ECM) made up of water, collagen, proteoglycans, along with various non-collagenous proteins and glycoproteins present in smaller quantities. Chondrocytes represent the sole cell type within cartilage and account for approximately 2-10% of the total cartilage volume. These cells synthesize cartilage matrix and are responsible for cartilage turnover which involves both degradation and synthesis. Cartilage is mainly composed of the proteins, collagen type II and Aggrecan. There are also other proteins in articular cartilage but in smaller quantities that play a role during matrix formation or regulate cell function. For example: type I, VI, IX, X and XI collagens, different types of non-collagenous proteins like fibronectin, cartilage oligomeric matrix protein (COMP) which make additional cross-linking and act as stabilizers of the collagen network and hyaluronan which is a main component of both synovial fluid and cartilage matrix and bonds aggrecan molecules together [231-233].

1.6.2 Cartilage biomarkers in joint destruction

The most abundant collagen type in the cartilage (around 90%) is type II collagen. It is only found in cartilage, which makes it a good marker for cartilage formation and degradation. The network that type-II collagen forms is pivotal for the morphology and stability of articular cartilage. The structure is made of three identical alpha chains that together make a triple helix. Single collagen molecules are crosslinked by other proteins in order to form larger collagen fibers. Type II collagen can be degraded enzymatically and generate different cleavage neoepitopes. These neoepitopes are released from cartilage matrix into body fluids such as SF, serum and urine and can be used to record cartilage degradation. The initial enzymatic cleavage of type II collagen by collagenases, metalloproteinases and cathepsin K, produces two fragments: a ¾ piece and a ¼ length fragment. As a result of this the triple helix unwinds and expose the new epitopes for detection and further cleavage by a variety of proteases [232, 234, 235].

C2C neoepitope

Type II collagen collagenase cleavage neoepitope (C2C) is the 'new' carboxyterminal end of the new fragment generated as a result of the primary cleavage of type II collagen by collagenases. Additional cleavage generates a smaller fragment consisting of forty-five amino acids which still contains the C2C epitope. C2C is used as a marker for cartilage degradation and turnover [236]. C2C concentrations in articular cartilage are increased in knee Osteoarthritis (OA) [237]. Among OA patients receiving biologic therapy, there was a correlation between serum C2C concentrations and radiographic assessments. Elevating C2C levels were associated with disease progression, while decreasing values were linked to disease remission [238, 239]. In OA patients with no radiographic signs of joint destruction, elevated urine (but not serum) C2C levels are observed [240]. In RA, high C2C levels suggested a progressive course of joint destruction. The serum C2C concentration was decreased after biologic therapy [238, 239]. In a study on JIA, it was seen that unlike adults

with RA, serum C2C levels were significantly lower in JIA patients compared to healthy controls which suggest an inhibition of cartilage turnover in these patients. However, the levels did not correlate with disease duration, with bone erosion score nor clinical measure of disease activity and damage. The median C2C level was notably higher in patients who experienced structural damage progression, as indicated by joint space narrowing (JSN), compared to those without such progression. Patients, who at the 6 months follow-up visit needed either initiation of methotrexate or addition of a biologic agent, had significantly increased levels of C2C compared to patients who did not need to change the treatment. The authors proposed that biomarkers indicating cartilage breakdown hold promise as potential indicators for predicting the progression of structural damage and the severity of the disease course [241].

ARGS-aggrecan neoepitope

Aggrecan is the largest and most abundant proteoglycan protein in articular cartilage. It is composed of mass of glycosaminoglycans (GAGs) (keratan sulfate and chondroitin sulfate). Many GAGs are bond to a central core-protein. Glycosaminoglycan chains are highly polar and thus attract water. Aggrecan molecules bind to hyaluronic acid and is stabilized by a link protein. In a study on JIA patients, it was noted that the concentration of GAG in SF from JIA patients was considerably lower when compared to SF from patients with OA, juvenile knee injury, and individuals with healthy knees. It has been observed that GAG concentrations are decreased in both OA and RA patients, and they do not correlate with radiographic scores. The levels were lower in inflamed RA and OA knees compared with non-inflamed ones [233, 242].

Cartilage oligomeric matrix protein (COMP)

Cartilage oligomeric matrix protein (COMP) is not specific for cartilage. It is synthesized by chondrocytes but also by other cell types including synovial cells and osteoblasts [243]. Cartilage degradation causes the release of COMP in synovial fluid but in synovial inflammation, COMP levels might increase in the serum as well [243, 244]. It has been reported that during early disease stages of RA, COMP levels increases but it decreases afterwards [245]. In RA, high initial values of COMP predicted the progression of joint destruction and biologic therapy (anti TNF-α therapy) correlated with a decrease in serum COMP [246, 247]. In JIA, decreased COMP levels compared with healthy children, has been observed. COMP values correlated negatively with inflammatory activity as indicated by CRP and the thrombocyte counts and were associated with reduced growth rate [248]. Serum COMP concentration showed a decrease in systemic JIA patient who has growth impairment, and it was increased after treatment with anti-IL-6 tocilizumab. The systemic inflammation observed in sJIA can potentially impact the process of cartilage turnover, which plays a critical role in children's growth. Elevated levels of COMP in the serum of healthy children are indicative of increased cartilage turnover during growth. Therefore, serum COMP concentrations could potentially serve as a marker signaling growth impairment in individuals with systemic JIA [249].

1.6.3 Bone biomarkers in joint destruction

Amino-terminal collagen crosslinks (NTX-I)

Type I collagen composes 90–95% of the bone extracellular matrix secreted by osteoblasts. Antibodies have been made to N-terminal and C-terminal (amino and carboxy) telopeptides that constitute cross-links. Amino-terminal collagen crosslinks (NTX-I) and carboxy-terminal collagen crosslinks (CTX-I) are used as biomarkers to detect bone turnover and

measure mainly bone resorption in the urine or serum. However, Type I collagen is also found in skin and tendons and thus it is not a specific biomarker for bone turnover [250]. In a study on JIA, it was observed that, JIA patients had elevated levels of CTX-I compared to the healthy controls. However, patients with radiographic progression had reduced levels of CTX-I compared to patients without structural damage progression. Decreased bone mass and bone turnover was observed in children with higher degrees of joint destruction [251].

Tartrate-resistant acid phosphatase (TRAP)

Tartrate-resistant acid phosphatase (TRAP) is an iron-containing enzyme commonly found in bone and the immune system. It plays a vital role in numerous biological functions, including skeletal growth, collagen synthesis and breakdown, cytokine generation by macrophages and dendritic cells, recruitment of macrophages, maturation of dendritic cells, and its involvement in the formation of Th1 immune responses [252]. Serum contains two TRAP isoforms, 5a and 5b, derived from post-translational modifications of a single gene product [253]. TRAP 5a is released by macrophages and dendritic cells, while TRAP 5b is predominantly secreted by activated osteoclasts. TRAP 5b serves as a biomarker to measure osteoclast count and bone resorption levels. Its diagnostic and prognostic potential extends to conditions such as osteoporosis, cancers with bone metastasis, and various metabolic and pathological bone disorders. Observations indicate that within the synovium near the cartilage-synovial boundary, TRAP-positive mononuclear and multinucleated cells are responsible for the production of MMP-2 and MMP-9. These cells are believed to play a significant role in the degradation of articular cartilage in patients with rheumatoid arthritis (RA) [254]. It is important to notice that elevated TRAP 5b is observed during normal bone growth in children as well [252, 255-257].

2 ETHICAL CONSIDERATIONS

In the pursuit of scientific knowledge and advancements in the field of pediatric rheumatology, our research projects involved the utilization of human materials, including blood, synovial fluid (SF) and urine obtained from biobanks, as well as personal information from patients and healthy donors. Ensuring the highest ethical standards and patient privacy was essential in our research.

Informed Consent and Patient Privacy

Before any sample collection, comprehensive informed consent was diligently obtained from the children involved in our studies and their parents and healthy donors respecting their autonomy and ensuring that they understood the nature and purpose of the research. This ethical foundation safeguards patient privacy and their rights as research participants.

Synovial Fluid Collection

It's crucial to emphasize that the collection of synovial fluid (SF) was exclusively performed on patients experiencing swollen joints and mobility issues. This procedure was essential for clinical diagnosis and was executed by qualified clinical doctors. Our approach prioritizes the patients' well-being, aligning with the principles of beneficence and non-maleficence in medical ethics.

Stringent Data Management

To protect patient data, all information was meticulously coded, rendering it anonymous and preventing any personal identification. This safeguard extended to details such as patient birthdates, disease onset dates, and treatments. To further secure data, access to the key for decoding was restricted to authorized personnel within the research group.

Data Handling and Sharing

We strictly adhered to data security protocols. Patient data was never shared via email or external communication channels to mitigate the risk of unintended data exposure. Additionally, electronic files containing sensitive information were safeguarded with Personal Identification Number (PIN) codes when necessary.

Transparency and Accountability

Transparency was of utmost importance in our research. We hosted informative events to relay the latest project developments to the patients and their families. During these sessions, we were committed to providing clear and open explanations of the research objectives, hypotheses, sample processing, and accomplishments. This transparency was accompanied by a platform for participants to ask questions and seek clarifications, ensuring accountability throughout the research process.

Voluntary Participation

It is imperative to underline that participation in our studies was entirely voluntary. Equally, the decision to withdraw from the research at any point was fully respected. Ethical approval was diligently secured for all sample collections and data gathering processes.

In summary, our research activities were conducted with the utmost respect for ethical standards and the well-being of the patients involved. Our commitment to transparency, data security, and patient privacy safeguards the ethical integrity of our studies and maintains the trust of our research participants.

3 AIMS OF THE THESIS

This thesis aimed to enhance our understanding of the disease mechanisms underlying Juvenile Idiopathic Arthritis (JIA) by delving into the genetic background of JIA and exploring specific immunological and biochemical biomarkers for improving diagnosis and prognosis of JIA. Comprising four distinct studies, this work collectively unveiled various aspects of JIA, forming a cohesive exploration of this complex condition. The specific aims were to:

1. Study I: Genetic Risk Factors and Autoantibodies in JIA

- Uncover SNP genetic risk polymorphysms associated with JIA through a genome-wide association analysis in a Swedish cohort.

- Explor genetic association of JIA with classical Human Leukocyte Antigen (HLA) haplotypes and explore their relationships with specific autoantibodies (ANA, RF, ACPA).

- Investigate potential interactions between genetic factors and autoantibody production, specifically ANA in JIA.

2. Study II: *LACC1* Variations in Non-Systemic JIA

- Investigate genetic variations at the *LACC1* locus in non-systemic JIA, a relationship that had not been previously explored.

- Replicate previous associations between *LACC1* polymorphisms and inflammatory bowel diseases (crohn's disease and ulcerative colitis) within a large Swedish cohort.

3. Study III: ACPAs in JIA

- Examine the prevalence of anti-citrullinated protein/peptide antibodies (ACPA), particularly anti-CCP antibodies, in Swedish JIA patients and healthy age-matched references.

- Identify ACPA reactivities in JIA patients and healthy children using a multiplex microarray platform.

- Investigate correlations between ACPAs, HLA SE, and clinical characteristics.

4. Study IV: Biomarkers of Joint Degradation in JIA

- Explore the levels of biomarkers associated with bone and cartilage degradation in Swedish JIA patients, healthy children, and those with acute knee injuries.

- Assess the utility of different biofluids (Plasma, SF and Urine) in detection of these biomarkers for early detection of joint damage in JIA.

- Investigate the impact of confounding factors such as age, gender, disease onset, disease duration, extent of joint involvement, and medication usage on biomarker levels.

These four studies collectively contribute to a deeper understanding of JIA, from genetic risk factors and autoantibodies to biomarkers for early joint degradation. The findings have implications for diagnostics, prognostics, and potential for refining JIA subtyping and treatment strategies in future.

4   RESULTS AND DISCUSSION

4.1   STUDY I: GENETIC ASSOCIATION OF ANTINUCLEAR ANTIBODIES WITH HLA IN JIA PATIENTS

Our study had a dual objective: firstly, to uncover new genetic risk factors linked to JIA through a genome-wide association analysis within a Swedish cohort. Secondly, to enhance our understanding of classical HLA genetic associations with JIA, while also exploring their relationships with specific autoantibodies (ANA, RF, and ACPA).

We examined a group of 329 JIA patients from Stockholm, Sweden and 728 healthy adult controls. Characteristics of the study participants is described in **Table 1, Study I**.

DNA was extracted by salting-out method and the Illumina OmniExpress array was used for genotyping, and we employed the SNP2HLA algorithm to estimate HLA alleles from our GWAS data.

Through our case-control analysis, we identified 12 single SNPs that showed strong associations with JIA at the genome-wide significance level. These SNPs were all situated on chromosome 6 within the Major Histocompatibility Complex (MHC) class II gene region (**Table 2, Study I**). Notably, the most significant SNP (rs28421666) was located near *HLA-DQA1* and *HLA-DRB1* genes (**Figure 1, Study I**).

Although high-throughput probe-based genotyping technologies have facilitated the comprehensive examination of DNA sequence variations in genome-wide association studies, they have faced challenges in accurately capturing variations within HLA genes. The limitations in resolution of probe-based methods for HLA genotyping is due to the exceptionally polymorphic nature of these genes. Alternative methods such as imputation

have been developed to infer classical alleles by utilizing SNP genotypes from existing GWAS data, providing an indirect approach to HLA typing [258].

Imputation is a statistical technique commonly employed in genetic studies to extrapolate and estimate genetic variations based on LD patterns among neighboring SNPs in genomic regions that have not undergone direct genotyping via GWAS. This method involves the comparison of established haplotypes from a reference panel which is a large dataset containing genotypic information on a wide range of genetic variants from a diverse population with the haplotypes derived from the study participants to fill in missing genetic data. By doing so, imputation allows for informed predictions regarding the genotypes of ungenotyped variants. The outcome is a more exhaustive understanding of the genetic diversity present within a given population [258].

Thus, to identify HLA alleles driving the genome-wide association signals on chromosome 6, classical HLA alleles in the major histocompatibility complex (MHC) region were imputed from GWAS SNP data (Table 3A, Study I) using Type 1 Diabetes Genetics Consortium (T1DGC) reference panel.

In the overall cohort of JIA, *HLA-DRB1*08:01, HLA-DQA1*04:01*, and *HLA-DQB1*04:02* were the alleles most strongly linked to an increased risk of JIA. When we looked at specific subtypes, these alleles were associated with oligoarthritis and RF-negative polyarthritis (Table 4, Study I). An extended *HLA-DRB1-DQA1-DQB1* haplotype encoding the DR8 and DQ4 molecules has consistently been implicated as conferring increased risk to JIA [54, 59, 259-263]. In subtype-specific association analysis these alleles were found particularly in oligoarthritis and RF-negative polyarthritis patients, which suggests similar genetic predisposition for these two subtypes as has been observed before [54, 59, 259-263].

Within the linkage disequilibrium (LD) of the *HLA-DRB1-DQA1-DQB1* haplotype, our analysis suggested that *HLA-DRB1*08* might be the primary allele associated with JIA susceptibility. The *HLA-DRB1*11* allele group also independently correlated with JIA, particularly in the oligoarthritis patient group. Additionally, *HLA-B*27:05* was notably associated with the enthesitis-related arthritis subtype. These findings underscore the importance of considering different JIA subtypes separately due to their unique genetic associations.

Our investigation unveiled a statistically significant connection between female sex, the presence of anti-nuclear antibodies (ANA) and specific HLA alleles (**Supplementary figure 2, Study I**). By comparing patients who carry the HLA risk allele *HLA-DRB1*08* to those who don't have it we observed that, *HLA-DRB1*08*-positive patients had significantly lower age at onset, higher frequency of females and were more often ANA-positive (**Table 5, Study I**). All RF-positive polyarthritis patients were *HLA-DRB1*08*-negative. Regarding *HLA-DRB1*11*, it was also observed that *HLA-DRB1*11*-positive patients had higher frequency of ANA antibodies.

By performing association analysis comparing ANA-positive patients to controls we observed that the ANA-positivity, associated significantly to *DRB1*08, DQA1*04* and *DQB1*04*, at a higher significance level (**Table 6A, supplementary Table 2 & Supplementary Figure 3A, Study I**). In contrast, the ANA-negative patients did not show any significant associations with the classical HLA alleles (**Supplementary Figure 3E Study I**). In addition, in ANA-positive patients, stepwise logistic regression conditional analysis identified *DRB1*11* allele as a separate significant signal (**Table 6B & Supplementary Figure 3B_D, Study I**) in the region. With further conditioning for both *DRB1*08* and *DRB1*11*, *DPB1*02* was found as an independent association allele. The

observed correlations between specific HLA alleles and ANA positivity suggest potential interactions between genetic factors and autoantibody production in JIA.

On the other hand, *DQB1*06*, *DRB1*15*, *DRB1*07*, *DRB1*04*, *DQA1*03*, *DQB1*02*, *C*06* and *A*03* alleles were protective among ANA-positive patients (FDR<0.05) **(Supplementary Table 2, Study I).**

Next, we performed additional comparison between ANA-positive and ANA-negative JIA patients **(Table 7, Study I).** It was observed that ANA-positive patients had a significantly higher percentage of *DRB1*08*, 39% vs 19% in ANA-negative patients (P=0.0016), *DQA1*04*, 38% in ANA-positive vs 18% in ANA-negative (P= 0.0016), *DQB1*04*, 36% in ANA-positive vs 18% in ANA-negative (P= 0.0016) and *DRB1*11*, 31% in ANA-positive vs 17% in ANA-negative (P= 0.032). The ANA-positive group also had higher frequency of females (80.5% vs 59.4%) than the ANA-negative group (P=0.0008) and age at disease onset was significantly lower in the ANA-positive group, 3 vs 8 years in the ANA-negative group (P= 0.0016). The majority of ANA-positive patients were in the oligoarthritis subtype group (88 patients, 61%) and RF-negative polyarthritis subtype group (37 patients, 25%). Additionally, 35 patients (13%) of ANA-positive JIA were distributed across other subtypes. sJIA and ERA subcategories were more frequently ANA negative.

We further used logistic regression to examine the relationship between key factors (*DRB1*08, DRB1*11*, gender, and age of onset) and the presence of ANA in JIA patients. Our results indicated that *DRB1*08*, *DRB1*11*, gender, and age of onset were all contributing to the risk of ANA positivity in JIA patients with different effect sizes **(Table 8, Study I)**. Among these factors, *DRB1*08, DRB1*11*, and female gender had the highest statistically significant B regression coefficients of 0.79, 0.77, and 0.75, respectively. In contrast, age of onset had a smaller yet still statistically significant coefficient of -0.01, suggesting its role in promoting an ANA-positive profile in JIA.

These results align with the categorization established by the Paediatric Rheumatology International Trials Organization (PRINTO), which recognizes a subgroup of arthritis referred to as 'early-onset ANA-positive JIA'. This category includes children with arthritis onset at ≤6 years of age, who exhibit at least two ANA titers of ≥1:160 and do not have any other identifiable form of JIA [18]. This classification emerged from studies demonstrating that children with JIA meeting the specified ANA threshold typically experienced an earlier age of onset (80% under 6 years), had fewer affected joints involved, a lower frequency of radiographic joint damage, and a higher incidence of uveitis compared to patients falling into the oligoarthritis, RF-negative polyarthritis, and psoriatic JIA subtypes who never tested positive for ANAs. They proposed that individuals with JIA who test positive for ANA constitute a specific and uniform subgroup, often classified within different subtypes regardless of the progression of joint disease and the manifestation of psoriatic features [32, 264, 265]. If validated through additional research, this suggestion has the potential to result in a more refined classification of JIA [266].

In conclusion, our study contributes to the growing body of research that highlights the complex interplay between genetics, autoimmunity, and clinical phenotypes in JIA. We have identified significant associations between specific HLA alleles, ANA positivity, and the age of onset in a Swedish JIA cohort. The group of JIA patients who tested positive for ANA exhibited stronger associations with *HLA-DRB1*08:01*, *HLA-DRB1*11*, and *HLA-DPB1*02*, suggesting a potential link between genetic factors and the production of ANA in JIA. These insights raise the possibility of using ANA positivity as a marker for refining JIA subtyping and treatment strategies, aligning with the proposal for an 'early-onset ANA-positive JIA' subtype as suggested by the Paediatric Rheumatology International Trials Organization (PRINTO) [18].

Our study has limitations, including a relatively small cohort size, potential population-specific effects, retrospective data collection, and the absence of ANA data in healthy individuals and some of the patients. Further research with larger and more diverse cohorts, prospective data collection, and a comprehensive understanding of ANA status in healthy populations is essential to validate and expand upon our findings. Additionally, investigating the clinical utility of ANA positivity in stratifying JIA subtypes and its implications for personalized treatment approaches remains an avenue for future exploration.

4.2 STUDY II: LACC1 POLYMORPHISMS IN INFLAMMATORY BOWEL DISEASE AND JUVENILE IDIOPATHIC ARTHRITIS [267]

Juvenile Idiopathic Arthritis is a heterogeneous condition resulting from the complex interplay between various genes and environmental factors. While it is primarily a polygenic disease, meaning that multiple genes contribute to its development, some rare cases of monogenic familial JIA have been documented. One such instance involved a recessively inherited rare missense mutation in the *LACC1* gene (Cys284Arg) reported in familial systemic JIA as well as in monogenic forms of Crohn's disease (CD) suggesting involvement of a dysregulated innate immunity in pathogenesis [76, 268]. Furthermore, common variants of *LACC1* had been found associated to inflammatory bowel disease (IBD) [269-271]. However, there were no previous reports on the potential association between common *LACC1* polymorphism and JIA, in particular non-systematic forms. Therefore, the aim of this study was to investigate genetic variations at the *LACC1* locus in non-systemic JIA for the first time and to replicate previous observed associations in a large well-established Swedish IBD case–control cohort in collaboration with other researchers at Karolinska Institutet who were working on IBD.

When this study was performed, the biological function of LACC1 was unknown but the gene *LACC1* encodes the enzyme laccase (multicopper oxidoreductase) domain–containing 1. Laccase is an enzyme with a wide-ranging ability to facilitate the oxidation of various substances, such as polyphenols, aromatic amines, and inorganic ions; it does not exhibit specificity in its catalytic action [272, 273].

To explore the candidate gene *LACC1*, we conducted genotyping on 11 SNPs. These SNPs were chosen due to their functional characteristics (involving coding variants) and tagging capabilities, as illustrated in **Figure 1**, **Study I**. Subsequently, we assessed their impact on disease susceptibility among a cohort of 3,855 individuals from Sweden. This group

comprised of 1,124 individuals with Crohn's disease (CD), 1,297 with ulcerative colitis (UC), 229 with non-systemic juvenile idiopathic arthritis (nsJIA), and 1,205 healthy controls all collected in Sweden. Characteristics of study participants are shown in **Table 1, Study I.**

In this study, we reported connections between *LACC1* variations and several conditions, including CD, UC, IBD, and nsJIA (**Table 2 & Table 3, Study I**). We observed significant associations between eight *LACC1* variants and CD. Additionally, six of these SNPs exhibited a significant association with an increased risk of UC in the Swedish study population. Notably, four out of the eleven chosen SNPs were also significantly linked to an elevated risk of nsJIA. Importantly, the genetic risk effects observed were consistent for CD, UC, and nsJIA, suggesting that similar underlying mechanisms involving *LACC1* polymorphisms likely contribute to the predisposition to these conditions.

These results build upon prior research, which had demonstrated the connection between *LACC1* variations and conditions such as sJIA, CD and potentially UC [76, 268, 270, 271, 274, 275]. The significant association of *LACC1* polymorphisms with conditions such as UD, CD and JIA, raises questions about potential shared molecular functions and pathways underlying the pathophysiology of these seemingly disparate diseases, despite them only sharing partial clinical characteristics. While UC and CD have been generally understood as conditions rooted in the dysregulated interplay between the immune system and the gut microbiota, emerging research provides some compelling insights about functional alterations within the gut microbiome of JIA patients as well [276, 277]. Notably, the composition of the microbiota in JIA patients exhibits characteristics that have been previously reported in various other diseases, including CD [277]. This discovery opens up the possibility that *LACC1* genetic variation might exert its influence on IBD (involving UC and CD), and JIA risk through shared mechanisms.

While the exact role of *LACC1* in the context of JIA and IBD remains unclear, recent studies have demonstrated the essential role of *LACC1* in macrophage functions such as phagocytosis and autophagy. Macrophages lacking *LACC1* exhibit a diminished capacity to engulf apoptotic bodies and bacteria compared to the control group [119] [120, 121]. Macrophages play a central role in the immune system by engulfing and removing cellular debris, pathogens, and apoptotic bodies as well as antigen presentation to T cells and they promote tissue homeostasis. In situations where phagocytosis and autophagy are impaired, the effective removal of phagocytosed bacteria and apoptotic bodies may be compromised [278]. Consequently, this leads to their transition into secondary necrosis, marked by membrane integrity loss. This, in turn, results in the release of proinflammatory mediators and danger-associated molecular patterns, as well as the exposure of intracellular self-antigens to the extracellular milieu. This inflammatory environment can further contribute to the activation of immune cells and exacerbate autoimmune responses [278-280]. On the other hand, primary necrosis is an abrupt event, leading to the unintentional release of largely unaltered danger-associated molecular patterns (DAMPs) and self-antigens. In contrast, in secondary necrosis multiple modifications occurs throughout the apoptotic process which induces alterations in autoantigens by caspases before they are released and exposed. Additionally, their modification can be further influenced by the surrounding extracellular microenvironment [279, 281]. These autoantigens will be presented by antigen presenting cells such as dendritic cells and in genetically susceptible individuals it can give rise to autoreactive T and B cells and result in the breakdown of tolerance and production of autoantibodies which are key factors in the development of autoimmunity [154, 282]. These autoantibodies generate immune complexes with their corresponding antigens, which are continually released as a result of ongoing secondary necrosis. These complexes are subsequently identified by the complement system, leading to an immunogenic removal process by circulating phagocytes. This underscores that the buildup of secondary necrotic

cells, resulting from impaired clearance of deceased cells, can initiate an adaptive immune response and contribute to persistent inflammation [279].

In summary, the presence of common susceptibility loci across different inflammatory diseases has contributed to our understanding of pathophysiological processes common in JIA and IBD. Understanding the role of LACC1 in JIA and the mechanisms by which *LACC1* polymorphisms impact the disease can provide insights into potential therapeutic strategies in future.

4.3 STUDY III: PRESENCE AND REACTIVITIES OF ANTIBODIES DIRECTED TO CITRULLINATED PEPTIDES IN A SWEDISH JIA COHORT

Although Antibodies against citrullinated proteins/peptides (ACPA) have been extensively studied in RA, the knowledge regarding JIA is limited and our study is the first Swedish study investigating ACPAs. The first aim in this study was to investigate the prevalence of anti-CCP antibodies in well-characterized Swedish JIA patients (n=334) and in healthy age-matched references (n=66) from the Stockholm region, the same region from which the JIA patient's samples were collected. These individuals did not have any inflammatory or autoimmune diseases. Anti-CCP antibodies were measured by the commercial CCP2 ELISA that measures IgG antibodies. Results showed that 6.2 % of the JIA patients were positive for CCP2 ELISA and had significantly higher levels of anti-CCP2 antibodies as compared to 2% in healthy references with lower levels of antibodies (P= 0.004) (**Figure 1, Study III**).

The anti-CCP-positive subset of patients were significantly older at disease onset, with a median age of 129 months (~11 years) *versus* 62 months (~5 years) in the anti-CCP-negative subset (p=0.002) and they were also more frequently positive for RF (~62% *versus* 1.6%, p<0.0001). Anti-CCP antibodies were detected in nine out of 13 (69%) RF-positive polyarthritis patients, while in the RF-negative polyarthritis subtype, only four out of 84 patients (4.7%) were positive. In the oligoarthritis subtype, six out of 170 patients (3.5%) were anti-CCP-positive, and in subtype of patients with undifferentiated arthritis, two out of four patients (50%) were positive. In the remaining subtypes, no anti-CCP reactivity was recorded.

Anti-CCP has been previously reported in a wide spectrum, ranging from around 2% to 24% in various studies and majority of positive patients have been reported to be RF-positive polyarthritis [180, 184, 195, 198, 283-285]. Although it has been reported in other subtypes of JIA as well and associated with a more aggressive disease [284, 286] [198].

In our study, close to 62% of the anti-CCP-positive JIA patients had at least one *HLA-DRB1 SE* allele, compared to ~33% in the anti-CCP-negative JIA patients (p= 0.002). There was no difference between the two groups in terms of disease duration. Upon analyzing the 100 JIA samples using the multiplex array, a statistically significant difference between the anti-CCP-positive JIA patients and anti-CCP-negative patients in terms of number of ACPA reactivities was observed (81% *versus* 11%, p<0.0001) (**Table 1, Study III**). Association between *HLA-DRB1 SE* allele and anti-CCP antibodies have been reported in both RA and JIA before [287] [198] and a previous study in JIA didn't observe that anti-CCP-positive patients had a longer disease duration compared with anti-CCP-negative patients as well [184].

Although the CCP2 ELISA is a widely used diagnostic tool for detecting ACPAs in clinical settings, it's important to note that the CCP2 peptide (or peptides) used in this assay is an artificial antigen. This means that its sequence doesn't correspond to any human proteins, and as a result, it is regarded as a substitute indicator for the actual *in vivo* antigenic targets of ACPAs. Thus, to investigate citrullinated peptides as targets of ACPAs, our next aim was to define ACPA reactivities present in JIA patients (n=100) and in healthy children reference group (n=191) using a multiplex microarray platform that is a tool to test for the presence of IgG antibodies against 15 citrullinated peptides. Peptides included: 15 citrullinated peptides derived from filaggrin (cfc1-cyc), fibrinogen (Fib$\alpha_{563-583}$, Fib$\alpha_{580-600}$, Fib$\alpha_{621-635}$, Fibα_{36-50}, Fibβ_{36-52}, Fibβ_{60-74}), vimentin (Vim$_{2-17}$, Vim$_{60-75}$), α-enolase (CEP-1) and heterogeneous nuclear ribonuclear protein (hnRNP) (Pept Z1, Pept Z2, Pept 1, Pept 5, Pept Bla26) (**Supplementary Table 1, Study III**). Anti-Cit-Vim60-75, anti-Cit-Fibβ60-74, anti-CEP-1, anti-Cit-Pept Z2 and anti-Pept 5, stood out as the most common antibodies among all JIA patients considered as one group. In anti-CCP-positive JIA patients, the highest reactivity was observed against Cit-Vim60-75, Anti-CEP-1, anti-Cit-Pept Z2, anti-Pept 5, and anti-Cit-Fibβ60-74 with prevalence ranging from 50% to 69% (**Table 2, Study III**). These specific peptides have been previously reported as the most prevalent in RA, although at a higher

frequency, ranging from 60% to 80% in the CCP2-positive group and 3% to 9% in the CCP2-negative group [288]. The prevalence of anti-citrullinated vimentin antibodies in JIA has been previously reported at five to nine percent [193, 195, 197], citrullinated fibrinogen at 32% and citrullinated α-enolase at 9% [194].

In our study, ACPA reactivities were detectable in a total of 11% of patients who tested negative for anti-CCP antibodies, although the prevalence of individual antibodies within this subtype was significantly lower. The most common reactivity in anti-CCP-negative group was observed with Cit-Pept Z2, Cit-Pept 5, anti-Cit-Vim60-75 and anti-CEP-1 with prevalence ranging from 1% to 4%.

Reactivity to Cit-Vim$_{60-75}$ was the only reactivity having a statistically significant association to *HLA-DRB1* SE (**Table 3, Study III**).

We next investigated co-occurrence of investigated ACPAs together (**Figure 2, Study III**). We observed that several peptides exhibited a statistically high correlation with each other, including CCP2, cfc1-cyc, Pept Z2, Fibβ60-74, CEP-1, Fibα621-635, Fibα563-583, and Pept Bla26 (phi ≥ 0.63). Additionally, Fibα36-50, Fibα621-635, Pept Bla26, and Fibα563-583 had a significantly high correlation together (phi ≥ 0.78). On the other hand, Cit-Pept 1 and Cit-Vim 2-17 had the lowest correlation with other peptides (phi < 0.5). These observations are in accordance with previous reports in RA [289] but similar study in JIA have not been done before.

The current study has certain limitations. The investigation of ACPA reactivity using a multiplex array was restricted to a specific subset of JIA patients. Consequently, the prevalence of ACPAs in the findings might be skewed towards patients who are anti-CCP positive, making it inappropriate to generalize these results to the entire JIA patient population. It is important to note that ACPAs have previously been associated with a more

severe and erosive form of the disease. Unfortunately, we did not possess the requisite data to perform this specific analysis. Additionally, longitudinal studies involving individuals with new or early-onset JIA will be essential for a deeper understanding of the role played by citrullinated autoantibodies in disease prognosis and joint damage.

In summary, our study uncovers diverse ACPA reactivities in a Swedish JIA cohort, with ACPA occurrence extending beyond RF-positive polyarthritis. *HLA-DRB1* SE alleles also pose a risk for various ACPA reactivities in childhood. Additionally, our findings highlight the potential of the multiplex assay to identify ACPA reactivity in some of the anti-CCP2-negative patients which may suggest common pathways and treatment responses in these patients.

Future research should explore ACPA profiles as potential diagnostic and prognostic biomarkers in subtypes of JIA and additionally, investigating the connection between ACPA profiles, disease activity, and the progression of early onset disease over the course of the condition. Further studies of ACPA-positive JIA patients may offer unique insights in pathological mechanisms underlying the disease, given their differences from adult ACPA-positive RA patients in terms of risk factors and age of onset.

4.4 STUDY IV: JUVENILE IDIOPATHIC ARTHRITIS PATIENTS HAVE A DISTINCT CARTILAGE AND BONE BIOMARKER PROFILE THAT DIFFERS FROM HEALTHY AND KNEE-INJURED CHILDREN [230]

In previous studies we attempted to study the genetic risk factors for JIA that have a causal role and we also discussed autoantibodies in JIA which could play a role in disease pathogenies. Nevertheless, JIA may develop to a degenerative joint disease. Currently, there are no established biomarkers that can reliably identify the early phases of joint degradation in JIA before it becomes visible through radiological means. The presence of such biomarkers could prove valuable for tracking treatment effectiveness, assessing the advancement of joint damage early on, and potentially foreseeing the course of the disease.

This conducted study was exploratory in nature and served as a foundation for hypothesis generation. Our principal objective was to examine the levels of various extensively researched biomarkers associated with bone and cartilage degradation in other investigated conditions involving joint degeneration, particularly OA [290] within our JIA cohort and compare these findings with data from healthy children and children experiencing acute knee injuries. Our secondary goal involved the assessment of suitable biofluids for the detection of degradation biomarkers in JIA. Additionally, we investigated the potential impact of confounders such as age, gender, disease onset, disease duration, the extent of joint involvement, and medication usage at the time of sample collection.

The degradation of cartilage was tracked through the measurement of the ARGS (aggrecanase-generated neoepitope of aggrecan), the C2C neoepitope of collagen type II, and cartilage oligomeric matrix protein (COMP) (non-collagenous protein in the extracellular matrix, which stabilizes the collagen network). In order to assess bone degradation,

measurements were taken for the N-terminal type I collagen cross-linked telopeptide (NTX-I) (degradation peptide from type I collagen) and tartrate-resistant acid phosphatase 5b (TRAP5b) (an enzyme expressed by bone resorbing osteoclasts). They were quantified in various biofluids using the ELISA method.

Samples of synovial fluid, plasma, and urine were collected in triplets, all obtained simultaneously from 29 patients diagnosed with JIA and 183 plasma samples from controls were sourced from a population-based cohort in the Stockholm region. To address the limitation of low sample volume, the healthy reference group was divided into three sub-cohorts, each comprising 61 individuals, with careful matching based on age and gender. Synovial fluid samples from a knee injury cohort, 41 juvenile patients were selected. Characteristics and demographics of the subject groups are shown in **Table I, study IV**.

Our investigation revealed that all biomarkers were detectable in all biofluids in all the studied juvenile cohorts. In comparison to the healthy reference group, the JIA group had statistically elevated plasma concentrations of the cartilage biomarkers ARGS-aggrecan, C2C, and COMP. This suggests a more pronounced degree of cartilage degradation in JIA compared to healthy references. Likewise, the plasma concentration of the bone biomarker TRAP5b was statistically higher in the JIA group, whereas plasma NTX-I levels were lower in JIA group compared to healthy controls (**Figure 1a-e and Supplementary Table S2, study IV**). A previous study had reported an elevated levels of a similar bone marker, C-terminal type I collagen cross-linked telopeptide (CTX-I) in plasma compared to healthy controls. However, they reported that the concentration of CTX-I was reduced in JIA patients with more severe joint destruction [251].

Compared to juvenile knee-injured patients, the synovial fluid concentrations of the cartilage biomarkers ARGS-aggrecan and COMP were lower in the JIA group, while the cartilage C2C and the bone TRAP5b markers were significantly higher (**Figure 1f-i, Supplementary**

Table S2). Lower levels of ARGS-aggrecan in JIA compared to juvenile knee-injured patients had been previously reported and was confirmed in this study. These results may indicate either a lower aggrecan turnover in JIA or a different molecular pathway for degradation. Our findings suggest that tissue degradation in JIA is distinct from that observed in knee injuries. Specifically, there is increased osteoclast activation and collagen degradation in JIA, whereas in juveniles with knee injuries, there is higher degradation of aggrecan and COMP. This highlights the presence of distinct molecular signatures between these two patient groups.

Furthermore, in the analysis of biomarkers for diagnosing JIA compared to healthy references or juvenile injuries, the area under the curve (AUC) analyses indicated that plasma concentrations of C2C and TRAP5b exhibited strong discriminatory power, with AUC values of 0.95 and 0.92, respectively, effectively distinguishing JIA from healthy references. However, the AUC values for other biomarkers were comparatively lower. Moreover, when considering synovial fluid concentrations, C2C and TRAP5b demonstrated even higher differentiating capabilities, with AUC values of 0.96 and 0.97, respectively, effectively distinguishing JIA from juvenile injuries (**Supplementary Table S3**).

We compared the levels of various biomarkers collected simultaneously in triple biofluids, including plasma, synovial fluid, and urine. Regarding the cartilage biomarkers, no significant difference in concentrations between synovial fluid and plasma was observed for ARGS-aggrecan. However, for COMP, the median level in synovial fluid was significantly higher than in plasma. C2C levels in synovial fluid and plasma were assessed using plates from two different lot numbers, and the observed inter-batch variation was substantial, making direct comparison unfeasible. As for the bone biomarker TRAP5b, the median plasma levels were slightly elevated in comparison to the synovial fluid levels (**Figure 2**).

Although, we did not detect any correlation between local and systemic levels of any of the cartilage markers, the two bone biomarkers, TRAP5b and NTX-I on the other hand displayed high positive correlations in plasma as well as in plasma versus urine samples (**Figure 2, Table II**). The lack of correlation between local and systemic levels of cartilage markers among the JIA patients in this study may be attributed to the possibility that systemic levels could be influenced by joints other than the one from which synovial fluid was obtained. It's important to note that these JIA patients had as many as 10 joints affected by arthritis at the time of sample collection, with a median of 2 affected joints. However, despite the range in the number of active joints, no correlation was found between the number of active joints and the levels of any biomarkers. This observation remained consistent even when comparing patients with only one affected joint to those with two or more affected joints.

For the JIA patients, we also studied the impact of possible confounding factors that could influence the levels of degradation products. We observed an inverse relationship between the age at sampling of JIA patients and the levels of C2C in both plasma and urine, as well as with the levels of TRAP5b in synovial fluid and plasma. No other biomarker demonstrated a correlation with the age in these patients, and none of the biomarkers exhibited a correlation with disease duration, as detailed in Table III. Age-dependent fluctuations in TRAP5b levels have been previously reported in healthy children, with the highest peak occurring during infancy and puberty, regardless of gender [291].

Regarding gender distinctions, female JIA patients displayed median plasma C2C levels that were 1.4 times higher and plasma COMP levels that were 1.3 times higher compared to the male patients. However, no other variations in biomarker levels were identified between the sexes, as indicated in Table IV. Furthermore, we examined potential effects on biomarker levels based on the number of affected joints at the time of sampling, disease duration at the time of sampling, and whether patients were undergoing treatment. Notably, none of the

biomarkers showed a correlation with disease duration. Furthermore, patients with two or more affected joints at the time of sampling exhibited a 1.9-fold higher concentration of urine C2C. However, no additional disparities in biomarker levels were observed when comparing individuals with one affected joint to those with multiple affected joints. Additionally, there was no correlation found between the number of affected joints and biomarker concentrations, as outlined in **Tables III** and **IV**. Moreover, no variations in biomarker concentrations were detected between JIA patients receiving medication treatments and those who were untreated, as evidenced in **Tables I and III**. Due to small sample size, we didn't compare the biomarkers in different JIA subtypes.

In this study, we have demonstrated that JIA patients exhibit elevated levels of biomarkers associated with bone and cartilage degradation in comparison to healthy children. These findings suggest the potential utility of these biomarkers as valuable clinical tools for prognostic or diagnostic purposes in the context of joint damage. Specifically, our analysis of plasma samples highlights the feasibility of distinguishing between JIA patients and healthy individuals with a high degree of specificity and sensitivity, particularly when assessing C2C and TRAP5b. We also investigated the impact of gender, disease duration, the number of affected joints, and medication on biomarker levels within the JIA group. Interestingly, these factors did not significantly influence biomarker levels. Furthermore, we observed a strong correlation between local and systemic levels of bone markers, whereas cartilage markers displayed either weak or no correlation between synovial fluid and plasma levels. This suggests that these bone markers are well-suited for systemic measurements.

Additionally, our data revealed a distinct molecular signature associated with joint destruction in JIA, offering valuable insights into the underlying destructive processes during disease progression. Future research studies should focus on establishing correlations between these biomarkers and radiological imaging findings, as well as their clinical

relevance in verifying joint destruction, thus further elucidating their potential clinical applications especially in early detection of joint destruction and response to therapy.

5 CONCLUDING REMARKS

To advance our knowledge of the intricacies of Juvenile Idiopathic Arthritis (JIA), this thesis has explored key aspects of the disease, from genetic risk factors to autoimmune responses, potential biomarkers, and distinct molecular signatures associated with joint degradation. The findings from these studies shed light on the multifaceted nature of JIA, ultimately providing valuable insights for both current clinical practice and future research directions.

Genetic Risk Factors and Autoantibodies

The first study delved into the genetic underpinnings of JIA, revealing notable associations between specific HLA alleles, such as *HLA-DRB1*08:01* and *HLA-DRB1*11*, and the presence of anti-nuclear antibodies (ANA). These findings emphasize the importance of understanding the genetic factors contributing to autoimmunity in JIA, with potential implications for a change in subtyping patients. However, it is imperative to acknowledge the limitations of the study, such as its relatively small cohort size, and call for larger, more diverse cohorts and comprehensive data collection regarding ANA status and uveitis manifestation in patients and healthy controls to validate and expand these findings.

In the second study, we explored the potential involvement of *LACC1* polymorphisms in non-systemic JIA. Notably, we discovered associations between *LACC1* variants and not only JIA but also crohn's disease and ulcerative colitis. These findings raise intriguing questions about shared molecular mechanisms underlying seemingly distinct inflammatory conditions. As the role of *LACC1* in JIA is still not fully understood, future research should delve deeper into

the molecular mechanisms and pathways connecting *LACC1* polymorphisms and disease risk.

Autoantibodies in JIA

The third study investigated understanding autoantibodies in JIA, particularly the presence of anti-citrullinated protein/peptide antibodies (ACPA). By investigating the prevalence of anti-CCP antibodies and ACPA reactivities, the study revealed broader presence of ACPAs, surpassing not only RF-positive polyarthritis but also transcending the limits of detection by commercial ELISA tests. These findings challenge established assumptions about ACPA distribution. Additionally, distinctive patterns emerge concerning the specific types of ACPAs. Furthermore, the link between *HLA-DRB1 SE* alleles and ACPA presence in childhood highlights the genetic predispositions underlying ACPAs in JIA.

Biomarkers for Joint Degradation

In the fourth study, we shifted our focus to the joint degradation in JIA, aiming to identify and validate potential biomarkers that could facilitate early detection and tracking of joint damage. Our findings suggest that the biomarkers for cartilage and bone degradation could serve as valuable tools for prognostic and diagnostic purposes. We highlighted the capability of C2C and TRAP5b to distinguish JIA from healthy individuals with high specificity and sensitivity. Additionally, the study unveiled a distinct molecular signature associated with joint destruction in JIA, providing insights into the underlying mechanisms during disease progression.

6 FUTURE PERSPECTIVES

While this thesis contributes new insights into JIA, there is ample room for future exploration and discovery. The next steps should involve:

1. Validation and Expansion: Large-scale studies with more diverse and extensive cohorts are essential to validate the findings presented here. Such studies will provide a more comprehensive understanding of the genetic and autoimmune factors at play in JIA.

2. Molecular Mechanisms: Further research is needed to elucidate the molecular mechanisms linking genetic risk factors, autoantibody development, and joint degradation. This deeper understanding will inform the generation of targeted treatments.

3. Clinical Implications: Future research should prioritize establishing links between identified genetics, autoantibodies, and their clinical importance to determine genuinely distinct patient subtypes that hold significance in both clinical practice and research investigations. Additionally, it's essential to explore associations between the identified biomarkers and radiological imaging results to validate their clinical relevance in early joint damage detection and treatment response.

4. Precision Medicine: The insights gained from genetic and autoantibody studies underscore the potential for precision medicine based on genetic, immunological, and biomarker profiles tailored to specific JIA subtypes in future.

5. Longitudinal Studies: Comprehensive, long-term studies are vital to understand the evolution of JIA over time and how these factors (specifically antibody levels and destruction biomarkers) impact and develop over the course of the disease.

In conclusion, this thesis signifies notable advancements in our ongoing pursuit to unravel the complexities of important pediatric autoimmune disease, JIA. These findings not only enhance our understanding of the characteristics of the disease but also set the stage for future research, offering the potential for improved diagnostics, treatment approaches, and, most importantly, enhanced well-being for children affected by JIA.

7 ACKNOWLEDGEMENTS

Throughout my years of pursuing my PhD studies, I've had the privilege of encountering numerous individuals, each remarkable in their own right. They've brought with them diverse personalities and distinct outlooks on science and life, which have profoundly influenced me and contributed to the growth of my scientific knowledge and interpersonal skills. While it's unfeasible to acknowledge everyone individually, I want to express my heartfelt appreciation to each person involved in this journey. This includes not only the patients and contributors to our studies but also the individuals at the Center for Molecular Medicine (CMM), Karolinska Institutet, and beyond. Without the collective efforts of all these individuals, this journey would not have been possible. I'm grateful for all the highs and the lows, the setbacks and comebacks, the blessings, and the lessons I've learned during my time at Karolinska Institutet. My deepest appreciation goes out to everyone who played a role in this journey.

First, I wish to convey my utmost gratitude to my supervisors:

My main supervisor, Helena Erlandsson Harris, thank you for letting me do my PhD studies in your lab. I appreciate the advice and guidance you've given me. I aspire to have acquired your qualities of organization, conciseness, clarity, assertiveness, and determination. I'd also like to express my appreciation for having me part of the National Clinical Research School in Chronic Inflammatory Diseases (NCRSCID). It was a great experience.

My co-supervisors:

Erik Sundberg, I want to express my gratitude for your contributions, especially for providing all the crucial clinical information for our studies. Your support and holistic clinical perspective have been invaluable throughout our projects. I also appreciate your passion for life and motivation for helping others. Your caring nature was evident in your guidance on how to make the most of weekends and summers as well as how to endure during the tough and challenging times. Your presence has meant a great deal to me, and I truly value your support and kindness.

Leonid Padyukov, I feel incredibly fortunate to have had you join me as my supervisor along this journey. Your belief in my abilities, even when I doubted myself, has been a driving force. I'm grateful for the balance you establish between granting independence and providing your steady support. Your patience and dedication in addressing every question,

no matter how trivial, have been invaluable. You are a source of wisdom, knowledge, encouragement, positive energy and support – qualities any student would wish for in a supervisor. What's more, your humility, open-mindedness, and down-to-earth manner has truly impressed me. In my view, you embody an academic human version of ChatGPT and much more. My gratitude knows no bounds.

A heartfelt thank you goes out to our collaborators and co-authors, Karin Lundberg, André Struglics, Ghazaleh (Aida) Assadi, Mauro D'Amato, Lars Alfredsson, Ingrid Kockum, Monika Hansson, Linda Mathsson for their invaluable contributions to our research projects.

My opponent Ellen Berit Nordal and the Examination Board Maja Jagodic, Liv Eidsmo, Göran Andersson for your time and consideration and the upcoming scientific discussion.

I want to extend my gratitude to all the members of the HMGB1 group. Hanna, who guided me through the early stages of my journey at CMM, Lena for her immense assistance with patient samples and her kindness and Lotta for her valuable knowledge and remarkable support with file maker system, patient samples, and her contagious positive energy and attitude. Cissi for leading the destruction project and for the nice work trip to Lund. Other HMGB1 group: Ulf, Karin, Lasse, Peter, Hanna, Henna, Mia, Agnes, Dimitria and Rebecka for all the discussions, talks and kindness.

Special thanks to Jan Alvar and Xingmei and all the fellow members of the National Clinical Research School in Chronic Inflammatory Diseases (NCRSCID) for their efforts in organizing and fostering a conducive and enjoyable atmosphere throughout our collaborative meetings and for fantastic people we met.

Special thanks to all the inspiring and supportive people at Karolinska. Lars Klareskog, Ingrid Lundberg, Anca Catrina, Per-Johan Jakobsson, Marie Wahren-Herlenius, Vivianne Malmström, Bob Harris and Michael Fored. Stina and Veronica for their invaluable administrative assistance, Lina for her warm and helpful attitude, Boel for the GWAS course, Sam for the engaging scientific and non-scientific conversations, Heidi for her contagious positive energy, which never fails to bring a smile to everyone's face. The dedicated technicians who played a crucial role in sample preparation and human lab work: Gloria, Barbro, Julia, Susana, and Lena I. To Viole, Lara, Rita, Vijay, Akilan, Priya, Natalia, and Olga for making our shared office experiences enjoyable. Other remarkable individuals I've had the pleasure of meeting and cherishing as dear friends, Joan, Miranda, Jorge, Johanna,

Thank you all for being a meaningful part of this chapter in my life's journey. Your presence and friendship mean the world to me.

In the end, I would like to acknowledge the assistance of the GPT-3.5 AI language model provided by OpenAI. This technology provided valuable contribution in editing and refining of this thesis, particularly given that English is not my native language. I want to emphasize that the core content and primary text of this thesis were entirely composed by me. It is the product of my independent and critical analysis of previously published data, as well as my own original research. The AI language model from OpenAI was exclusively employed to enhance the text's clarity and style, with a strict commitment to preserving the original meaning. It had no impact on the factual content, or the conclusions presented in this thesis. I take full responsibility for this work and assert my sole authorship of the text.

Kind Regards,

Raya

8 REFERENCES

1. Rebane, K., et al., *Disability and health-related quality of life are associated with restricted social participation in young adults with juvenile idiopathic arthritis.* Scand J Rheumatol, 2019. **48**(2): p. 105-113.
2. Oliveira Ramos, F., et al., *Health-related quality of life and disability in adults with juvenile idiopathic arthritis: comparison with adult-onset rheumatic diseases.* RMD Open, 2021. **7**(3).
3. Berntson, L., et al., *Incidence of juvenile idiopathic arthritis in the Nordic countries. A population based study with special reference to the validity of the ILAR and EULAR criteria.* J Rheumatol, 2003. **30**(10): p. 2275-82.
4. Thierry, S., et al., *Prevalence and incidence of juvenile idiopathic arthritis: a systematic review.* Joint Bone Spine, 2014. **81**(2): p. 112-7.
5. Cardoso, I., et al., *Age and Sex Specific Trends in Incidence of Juvenile Idiopathic Arthritis in Danish Birth Cohorts from 1992 to 2002: A Nationwide Register Linkage Study.* Int J Environ Res Public Health, 2021. **18**(16).
6. Petty, R.E., et al., *International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001.* J Rheumatol, 2004. **31**(2): p. 390-2.
7. Mahmud, S.A. and B.A. Binstadt, *Autoantibodies in the Pathogenesis, Diagnosis, and Prognosis of Juvenile Idiopathic Arthritis.* Front Immunol, 2018. **9**: p. 3168.
8. Ombrello, M.J., et al., *Genetic architecture distinguishes systemic juvenile idiopathic arthritis from other forms of juvenile idiopathic arthritis: clinical and therapeutic implications.* Ann Rheum Dis, 2017. **76**(5): p. 906-913.
9. Saurenmann, R.K., et al., *Risk factors for development of uveitis differ between girls and boys with juvenile idiopathic arthritis.* Arthritis Rheum, 2010. **62**(6): p. 1824-8.
10. Gowdie, P.J. and S.M. Tse, *Juvenile idiopathic arthritis.* Pediatr Clin North Am, 2012. **59**(2): p. 301-27.
11. Ravelli, A. and A. Martini, *Juvenile idiopathic arthritis.* Lancet, 2007. **369**(9563): p. 767-778.
12. Kasapcopur, O., et al., *Diagnostic accuracy of anti-cyclic citrullinated peptide antibodies in juvenile idiopathic arthritis.* Ann Rheum Dis, 2004. **63**(12): p. 1687-9.
13. Gurion, R., T.J. Lehman, and L.N. Moorthy, *Systemic arthritis in children: a review of clinical presentation and treatment.* Int J Inflam, 2012. **2012**: p. 271569.
14. Mellins, E.D., C. Macaubas, and A.A. Grom, *Pathogenesis of systemic juvenile idiopathic arthritis: some answers, more questions.* Nat Rev Rheumatol, 2011. **7**(7): p. 416-26.
15. Singh-Grewal, D., et al., *Predictors of disease course and remission in systemic juvenile idiopathic arthritis: significance of early clinical and laboratory features.* Arthritis Rheum, 2006. **54**(5): p. 1595-601.
16. Foll, D., H. Wittkowski, and C. Hinze, *[Still's disease as biphasic disorder : Current knowledge on pathogenesis and novel treatment approaches].* Z Rheumatol, 2020. **79**(7): p. 639-648.
17. Martini, A., *It is time to rethink juvenile idiopathic arthritis classification and nomenclature.* Ann Rheum Dis, 2012. **71**(9): p. 1437-9.
18. Martini, A., et al., *Toward New Classification Criteria for Juvenile Idiopathic Arthritis: First Steps, Pediatric Rheumatology International Trials Organization International Consensus.* J Rheumatol, 2019. **46**(2): p. 190-197.

19. Kimura, Y., et al., *Pulmonary hypertension and other potentially fatal pulmonary complications in systemic juvenile idiopathic arthritis.* Arthritis Care Res (Hoboken), 2013. **65**(5): p. 745-52.

20. Grom, A.A., A. Horne, and F. De Benedetti, *Macrophage activation syndrome in the era of biologic therapy.* Nat Rev Rheumatol, 2016. **12**(5): p. 259-68.

21. Huang, Z., et al., *mTORC1 links pathology in experimental models of Still's disease and macrophage activation syndrome.* Nat Commun, 2022. **13**(1): p. 6915.

22. Nies, J.F., U. Schneider, and M. Krusche, *Rare, rarer, lung involvement in adult-onset Still's disease: A mini-review.* Front Med (Lausanne), 2022. **9**: p. 989777.

23. Bracaglia, C., et al., *Elevated circulating levels of interferon-gamma and interferon-gamma-induced chemokines characterise patients with macrophage activation syndrome complicating systemic juvenile idiopathic arthritis.* Ann Rheum Dis, 2017. **76**(1): p. 166-172.

24. Weiss, E.S., et al., *Interleukin-18 diagnostically distinguishes and pathogenically promotes human and murine macrophage activation syndrome.* Blood, 2018. **131**(13): p. 1442-1455.

25. Cheema, G.S. and F.P. Quismorio, Jr., *Pulmonary involvement in adult-onset Still's disease.* Curr Opin Pulm Med, 1999. **5**(5): p. 305-9.

26. Canna, S.W. and E.M. Behrens, *Making sense of the cytokine storm: a conceptual framework for understanding, diagnosing, and treating hemophagocytic syndromes.* Pediatr Clin North Am, 2012. **59**(2): p. 329-44.

27. Nigrovic, P.A. and P.H. White, *Care of the adult with juvenile rheumatoid arthritis.* Arthritis Rheum, 2006. **55**(2): p. 208-16.

28. Polito, C., et al., *Growth retardation in non-steroid treated juvenile rheumatoid arthritis.* Scand J Rheumatol, 1997. **26**(2): p. 99-103.

29. Minden, K., *Adult outcomes of patients with juvenile idiopathic arthritis.* Horm Res, 2009. **72 Suppl 1**: p. 20-5.

30. Sandborg, C., et al., *Candidate early predictors for progression to joint damage in systemic juvenile idiopathic arthritis.* J Rheumatol, 2006. **33**(11): p. 2322-9.

31. Nigrovic, P.A., *Review: is there a window of opportunity for treatment of systemic juvenile idiopathic arthritis?* Arthritis Rheumatol, 2014. **66**(6): p. 1405-13.

32. Martini, A., *Are the number of joints involved or the presence of psoriasis still useful tools to identify homogeneous disease entities in juvenile idiopathic arthritis?* J Rheumatol, 2003. **30**(9): p. 1900-3.

33. Nigrovic, P.A., S. Raychaudhuri, and S.D. Thompson, *Review: Genetics and the Classification of Arthritis in Adults and Children.* Arthritis Rheumatol, 2018. **70**(1): p. 7-17.

34. Martini, A., et al., *Juvenile idiopathic arthritis.* Nat Rev Dis Primers, 2022. **8**(1): p. 5.

35. Ravelli, A. and A. Martini, *Juvenile idiopathic arthritis.* Lancet, 2007. **369**(9563): p. 767-78.

36. Hayward, K. and C.A. Wallace, *Recent developments in anti-rheumatic drugs in pediatrics: treatment of juvenile idiopathic arthritis.* Arthritis Res Ther, 2009. **11**(1): p. 216.

37. Reiff, A., et al., *Evaluation of the comparative efficacy and tolerability of rofecoxib and naproxen in children and adolescents with juvenile rheumatoid arthritis: a 12-week randomized controlled clinical trial with a 52-week open-label extension.* J Rheumatol, 2006. **33**(5): p. 985-95.

38. Lanni, S., et al., *Outcome and predicting factors of single and multiple intra-articular corticosteroid injections in children with juvenile idiopathic arthritis.* Rheumatology (Oxford), 2011. **50**(9): p. 1627-34.

39.   Ravelli, A., et al., *Glucocorticoids in paediatric rheumatology.* Clin Exp Rheumatol, 2011. **29**(5 Suppl 68): p. S148-52.

40.   Buer, J.K., *A history of the term "DMARD".* Inflammopharmacology, 2015. **23**(4): p. 163-71.

41.   Gutierrez-Suarez, R. and R. Burgos-Vargas, *The use of methotrexate in children with rheumatic diseases.* Clin Exp Rheumatol, 2010. **28**(5 Suppl 61): p. S122-7.

42.   Ravelli, A., et al., *Radiologic progression in patients with juvenile chronic arthritis treated with methotrexate.* J Pediatr, 1998. **133**(2): p. 262-5.

43.   Ruperto, N. and A. Martini, *Emerging drugs to treat juvenile idiopathic arthritis.* Expert Opin Emerg Drugs, 2011. **16**(3): p. 493-505.

44.   Pascual, V., et al., *Role of interleukin-1 (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade.* J Exp Med, 2005. **201**(9): p. 1479-86.

45.   Lovell, D.J., et al., *Adalimumab with or without methotrexate in juvenile rheumatoid arthritis.* N Engl J Med, 2008. **359**(8): p. 810-20.

46.   Quartier, P., 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): p. 747-54.

47.   Weiss, J.E. and N.T. Ilowite, *Juvenile idiopathic arthritis.* Rheum Dis Clin North Am, 2007. **33**(3): p. 441-70, vi.

48.   Horton, D.B. and S. Shenoi, *Review of environmental factors and juvenile idiopathic arthritis.* Open Access Rheumatol, 2019. **11**: p. 253-267.

49.   Zhao, W., C. Li, and J. Deng, *A systematic review and meta-analysis of environmental factors associated with juvenile idiopathic arthritis.* Int J Rheum Dis, 2023. **26**(7): p. 1235-1247.

50.   Clarke, S.L.N., et al., *Moving from nature to nurture: a systematic review and meta-analysis of environmental factors associated with juvenile idiopathic arthritis.* Rheumatology (Oxford), 2022. **61**(2): p. 514-530.

51.   Savolainen, A., et al., *Magnitude of the genetic component in juvenile idiopathic arthritis.* Ann Rheum Dis, 2000. **59**(12): p. 1001.

52.   Ansell, B.M., E.G. Bywaters, and J.S. Lawrence, *Familial aggregation and twin studies in Still's disease. Juvenile chronic polyarthritis.* Rheumatology, 1969. **2**: p. 37-61.

53.   Hinks, A., et al., *Investigation of rheumatoid arthritis susceptibility loci in juvenile idiopathic arthritis confirms high degree of overlap.* Ann Rheum Dis, 2012. **71**(7): p. 1117-21.

54.   Hinks, A., et al., *Dense genotyping of immune-related disease regions identifies 14 new susceptibility loci for juvenile idiopathic arthritis.* Nat Genet, 2013. **45**(6): p. 664-9.

55.   McIntosh, L.A., et al., *Genome-Wide Association Meta-Analysis Reveals Novel Juvenile Idiopathic Arthritis Susceptibility Loci.* Arthritis Rheumatol, 2017. **69**(11): p. 2222-2232.

56.   Hinks, A., et al., *Fine-mapping the MHC locus in juvenile idiopathic arthritis (JIA) reveals genetic heterogeneity corresponding to distinct adult inflammatory arthritic diseases.* Ann Rheum Dis, 2016.

57.   Thompson, S.D., et al., *The susceptibility loci juvenile idiopathic arthritis shares with other autoimmune diseases extend to PTPN2, COG6, and ANGPT1.* Arthritis Rheum, 2010. **62**(11): p. 3265-76.

58.   Prahalad, S. and D.N. Glass, *A comprehensive review of the genetics of juvenile idiopathic arthritis.* Pediatr Rheumatol Online J, 2008. **6**: p. 11.

59.   Hollenbach, J.A., et al., *Juvenile idiopathic arthritis and HLA class I and class II interactions and age-at-onset effects.* Arthritis Rheum, 2010. **62**(6): p. 1781-91.

60. Murray, K.J., et al., *Age-specific effects of juvenile rheumatoid arthritis-associated HLA alleles.* Arthritis Rheum, 1999. **42**(9): p. 1843-53.

61. McCarthy, M.I., et al., *Genome-wide association studies for complex traits: consensus, uncertainty and challenges.* Nat Rev Genet, 2008. **9**(5): p. 356-69.

62. Bush, W.S. and J.H. Moore, *Chapter 11: Genome-wide association studies.* PLoS Comput Biol, 2012. **8**(12): p. e1002822.

63. De Silvestri, A., et al., *HLA-DRB1 alleles and juvenile idiopathic arthritis: Diagnostic clues emerging from a meta-analysis.* Autoimmun Rev, 2017. **16**(12): p. 1230-1236.

64. Thomson, W., et al., *Juvenile idiopathic arthritis classified by the ILAR criteria: HLA associations in UK patients.* Rheumatology (Oxford), 2002. **41**(10): p. 1183-9.

65. Hinks, A., et al., *Fine-mapping the MHC locus in juvenile idiopathic arthritis (JIA) reveals genetic heterogeneity corresponding to distinct adult inflammatory arthritic diseases.* Ann Rheum Dis, 2017. **76**(4): p. 765-772.

66. Smerdel, A., et al., *Juvenile idiopathic arthritis (JIA) is primarily associated with HLA-DR8 but not DQ4 on the DR8-DQ4 haplotype.* Ann Rheum Dis, 2002. **61**(4): p. 354-7.

67. Ombrello, M.J., et al., *HLA-DRB1*11 and variants of the MHC class II locus are strong risk factors for systemic juvenile idiopathic arthritis.* Proc Natl Acad Sci U S A, 2015. **112**(52): p. 15970-5.

68. Hinks, A., 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): p. 1694-9.

69. Remmers, E.F., et al., *STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus.* N Engl J Med, 2007. **357**(10): p. 977-86.

70. Hinks, A., et al., *Association of the IL2RA/CD25 gene with juvenile idiopathic arthritis.* Arthritis Rheum, 2009. **60**(1): p. 251-7.

71. Prahalad, S., et al., *Variants in TNFAIP3, STAT4, and C12orf30 loci associated with multiple autoimmune diseases are also associated with juvenile idiopathic arthritis.* Arthritis Rheum, 2009. **60**(7): p. 2124-30.

72. Hinks, A., et al., *Association of the CCR5 gene with juvenile idiopathic arthritis.* Genes Immun, 2010. **11**(7): p. 584-9.

73. Moroldo, M.B., et al., *Juvenile rheumatoid arthritis affected sibpairs: extent of clinical phenotype concordance.* Arthritis Rheum, 2004. **50**(6): p. 1928-34.

74. Prahalad, S., et al., *Twins concordant for juvenile rheumatoid arthritis.* Arthritis Rheum, 2000. **43**(11): p. 2611-2.

75. Hou, X., et al., *The Multi-Omics Architecture of Juvenile Idiopathic Arthritis.* Cells, 2020. **9**(10).

76. Wakil, S.M., et al., *Association of a mutation in LACC1 with a monogenic form of systemic juvenile idiopathic arthritis.* Arthritis Rheumatol, 2015. **67**(1): p. 288-95.

77. Kallinich, T., et al., *Juvenile arthritis caused by a novel FAMIN (LACC1) mutation in two children with systemic and extended oligoarticular course.* Pediatr Rheumatol Online J, 2016. **14**(1): p. 63.

78. Karacan, I., et al., *LACC1 Gene Defects in Familial Form of Juvenile Arthritis.* J Rheumatol, 2018. **45**(5): p. 726-728.

79. Rabionet, R., et al., *Biallelic loss-of-function LACC1/FAMIN Mutations Presenting as Rheumatoid Factor-Negative Polyarticular Juvenile Idiopathic Arthritis.* Sci Rep, 2019. **9**(1): p. 4579.

80. Assadi, G., et al., *Functional Analyses of the Crohn's Disease Risk Gene LACC1.* PLoS One, 2016. **11**(12): p. e0168276.

81. Liu, H., et al., *Discovery of six new susceptibility loci and analysis of pleiotropic effects in leprosy.* Nat Genet, 2015. **47**(3): p. 267-71.

82. Twilt, M., et al., *Joint cartilage thickness and automated determination of bone age and bone health in juvenile idiopathic arthritis.* Pediatr Rheumatol Online J, 2017. **15**(1): p. 63.

83. Zaripova, L.N., et al., *Juvenile idiopathic arthritis: from aetiopathogenesis to therapeutic approaches.* Pediatr Rheumatol Online J, 2021. **19**(1): p. 135.

84. Swidrowska-Jaros, J. and E. Smolewska, *A fresh look at angiogenesis in juvenile idiopathic arthritis.* Cent Eur J Immunol, 2018. **43**(3): p. 325-330.

85. Wojdas, M., K. Dabkowska, and K. Winsz-Szczotka, *Alterations of Extracellular Matrix Components in the Course of Juvenile Idiopathic Arthritis.* Metabolites, 2021. **11**(3).

86. Macaubas, C., et al., *Oligoarticular and polyarticular JIA: epidemiology and pathogenesis.* Nat Rev Rheumatol, 2009. **5**(11): p. 616-26.

87. Spelling, P., et al., *Osteoprotegerin/RANKL system imbalance in active polyarticular-onset juvenile idiopathic arthritis: a bone damage biomarker?* Scand J Rheumatol, 2008. **37**(6): p. 439-44.

88. Stagi, S., et al., *Bone mass and quality in patients with juvenile idiopathic arthritis: longitudinal evaluation of bone-mass determinants by using dual-energy x-ray absorptiometry, peripheral quantitative computed tomography, and quantitative ultrasonography.* Arthritis Res Ther, 2014. **16**(2): p. R83.

89. Cui, A., et al., *Gene Expression Deconvolution for Uncovering Molecular Signatures in Response to Therapy in Juvenile Idiopathic Arthritis.* PLoS One, 2016. **11**(5): p. e0156055.

90. Wouters, C.H., J.L. Ceuppens, and E.A. Stevens, *Different circulating lymphocyte profiles in patients with different subtypes of juvenile idiopathic arthritis.* Clin Exp Rheumatol, 2002. **20**(2): p. 239-48.

91. Jones, E.Y., et al., *MHC class II proteins and disease: a structural perspective.* Nat Rev Immunol, 2006. **6**(4): p. 271-82.

92. Wedderburn, L.R., et al., *Selective recruitment of polarized T cells expressing CCR5 and CXCR3 to the inflamed joints of children with juvenile idiopathic arthritis.* Arthritis Rheum, 2000. **43**(4): p. 765-74.

93. Nistala, K., et al., *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): p. 875-87.

94. Wu, S.A., et al., *Persistent improper upregulation of Th17 and TReg cells in patients with juvenile idiopathic arthritis.* J Microbiol Immunol Infect, 2016. **49**(3): p. 402-8.

95. Berntson, L., et al., *Incidence of juvenile idiopathic arthritis in the Nordic countries. A population based study with special reference to the validity of the ILAR and EULAR criteria.* J Rheumatol, 2003. **30**(10): p. 2275-82.

96. Luger, D., et al., *Either a Th17 or a Th1 effector response can drive autoimmunity: conditions of disease induction affect dominant effector category.* J Exp Med, 2008. **205**(4): p. 799-810.

97. Liu, X., et al., *Intracellular MHC class II molecules promote TLR-triggered innate immune responses by maintaining activation of the kinase Btk.* Nat Immunol, 2011. **12**(5): p. 416-24.

98. Andreae, S., S. Buisson, and F. Triebel, *MHC class II signal transduction in human dendritic cells induced by a natural ligand, the LAG-3 protein (CD223).* Blood, 2003. **102**(6): p. 2130-7.

99. Trede, N.S., R.S. Geha, and T. Chatila, *Transcriptional activation of IL-1 beta and tumor necrosis factor-alpha genes by MHC class II ligands.* J Immunol, 1991. **146**(7): p. 2310-5.

100. Watts, R.A., et al., *Oxford Textbook of Rheumatology*. 2014, Oxford, UNKNOWN: OUP Oxford.

101. Jarvis, J.N., et al., *Gene expression profiling in neutrophils from children with polyarticular juvenile idiopathic arthritis.* Arthritis Rheum, 2009. **60**(5): p. 1488-95.

102. Corcione, A., et al., *Phenotypic and functional characterization of switch memory B cells from patients with oligoarticular juvenile idiopathic arthritis.* Arthritis Res Ther, 2009. **11**(5): p. R150.

103. Morbach, H., et al., *The kappa immunoglobulin light chain repertoire of peripheral blood B cells in patients with juvenile rheumatoid arthritis.* Mol Immunol, 2008. **45**(14): p. 3840-6.

104. Morbach, H., et al., *Activated memory B cells may function as antigen-presenting cells in the joints of children with juvenile idiopathic arthritis.* Arthritis Rheum, 2011. **63**(11): p. 3458-66.

105. Wiegering, V., H.J. Girschick, and H. Morbach, *B-cell pathology in juvenile idiopathic arthritis.* Arthritis, 2010. **2010**: p. 759868.

106. Pardeo, M., C. Bracaglia, and F. De Benedetti, *Systemic juvenile idiopathic arthritis: New insights into pathogenesis and cytokine directed therapies.* Best Pract Res Clin Rheumatol, 2017. **31**(4): p. 505-516.

107. Holzinger, D., D. Foell, and C. Kessel, *The role of S100 proteins in the pathogenesis and monitoring of autoinflammatory diseases.* Mol Cell Pediatr, 2018. **5**(1): p. 7.

108. Wittkowski, H., et al., *S100A12 is a novel molecular marker differentiating systemic-onset juvenile idiopathic arthritis from other causes of fever of unknown origin.* Arthritis Rheum, 2008. **58**(12): p. 3924-31.

109. Frosch, M. and J. Roth, *New insights in systemic juvenile idiopathic arthritis--from pathophysiology to treatment.* Rheumatology (Oxford), 2008. **47**(2): p. 121-5.

110. Silva, J.R. and I. Brito, *Systemic juvenile idiopathic arthritis versus adult-onset Still s disease: the pertinence of changing the current classification criteria.* Acta Reumatol Port, 2020. **45**(2): p. 150-151.

111. Brown, R.A., et al., *Neutrophils From Children With Systemic Juvenile Idiopathic Arthritis Exhibit Persistent Proinflammatory Activation Despite Long-Standing Clinically Inactive Disease.* Front Immunol, 2018. **9**: p. 2995.

112. Kessel, C., C.M. Hedrich, and D. Foell, *Innately Adaptive or Truly Autoimmune: Is There Something Unique About Systemic Juvenile Idiopathic Arthritis?* Arthritis Rheumatol, 2020. **72**(2): p. 210-219.

113. Zielinski, C.E., et al., *Pathogen-induced human TH17 cells produce IFN-gamma or IL-10 and are regulated by IL-1beta.* Nature, 2012. **484**(7395): p. 514-8.

114. Omoyinmi, E., et al., *Th1 and Th17 cell subpopulations are enriched in the peripheral blood of patients with systemic juvenile idiopathic arthritis.* Rheumatology (Oxford), 2012. **51**(10): p. 1881-6.

115. Record, J.L., T. Beukelman, and R.Q. Cron, *Combination therapy of abatacept and anakinra in children with refractory systemic juvenile idiopathic arthritis: a retrospective case series.* J Rheumatol, 2011. **38**(1): p. 180-1.

116. Ruperto, N., et al., *Abatacept in children with juvenile idiopathic arthritis: a randomised, double-blind, placebo-controlled withdrawal trial.* Lancet, 2008. **372**(9636): p. 383-91.

117. Cader, M.Z., et al., *C13orf31 (FAMIN) is a central regulator of immunometabolic function.* Nat Immunol, 2016. **17**(9): p. 1046-56.

118. Lahiri, A., et al., *Human LACC1 increases innate receptor-induced responses and a LACC1 disease-risk variant modulates these outcomes.* Nat Commun, 2017. **8**: p. 15614.

119. Omarjee, O., et al., *LACC1 deficiency links juvenile arthritis with autophagy and metabolism in macrophages.* J Exp Med, 2021. **218**(3).

120. Bonilla, D.L., et al., *Autophagy regulates phagocytosis by modulating the expression of scavenger receptors.* Immunity, 2013. **39**(3): p. 537-47.

121. Martinez, J., et al., *Microtubule-associated protein 1 light chain 3 alpha (LC3)-associated phagocytosis is required for the efficient clearance of dead cells.* Proc Natl Acad Sci U S A, 2011. **108**(42): p. 17396-401.

122. Nguyen, T.B., et al., *DGAT1-Dependent Lipid Droplet Biogenesis Protects Mitochondrial Function during Starvation-Induced Autophagy.* Dev Cell, 2017. **42**(1): p. 9-21 e5.

123. Abraham, C. and J.H. Cho, *Inflammatory bowel disease.* N Engl J Med, 2009. **361**(21): p. 2066-78.

124. Abraham, C. and R. Medzhitov, *Interactions between the host innate immune system and microbes in inflammatory bowel disease.* Gastroenterology, 2011. **140**(6): p. 1729-37.

125. Lassen, K.G., et al., *Atg16L1 T300A variant decreases selective autophagy resulting in altered cytokine signaling and decreased antibacterial defense.* Proc Natl Acad Sci U S A, 2014. **111**(21): p. 7741-6.

126. Dhillon, S.S., et al., *Variants in nicotinamide adenine dinucleotide phosphate oxidase complex components determine susceptibility to very early onset inflammatory bowel disease.* Gastroenterology, 2014. **147**(3): p. 680-689 e2.

127. Dallocchio, A., et al., *Occurrence of inflammatory bowel disease during treatment of juvenile idiopathic arthritis with etanercept: a French retrospective study.* Rheumatology (Oxford), 2010. **49**(9): p. 1694-8.

128. Barthel, D., et al., *Inflammatory Bowel Disease in Juvenile Idiopathic Arthritis Patients Treated with Biologics.* J Rheumatol, 2015. **42**(11): p. 2160-5.

129. Maller, J., et al., *Inflammatory Bowel Disease in Children With Systemic Juvenile Idiopathic Arthritis.* J Rheumatol, 2021. **48**(4): p. 567-574.

130. Verazza, S., et al., *Disease status, reasons for discontinuation and adverse events in 1038 Italian children with juvenile idiopathic arthritis treated with etanercept.* Pediatr Rheumatol Online J, 2016. **14**(1): p. 68.

131. van Straalen, J.W., et al., *Increased incidence of inflammatory bowel disease on etanercept in juvenile idiopathic arthritis regardless of concomitant methotrexate use.* Rheumatology (Oxford), 2022. **61**(5): p. 2104-2112.

132. van Dijken, T.D., et al., *Development of inflammatory bowel disease in patients with juvenile idiopathic arthritis treated with etanercept.* J Rheumatol, 2011. **38**(7): p. 1441-6.

133. Fragoulis, G.E., et al., *Inflammatory bowel diseases and spondyloarthropathies: From pathogenesis to treatment.* World J Gastroenterol, 2019. **25**(18): p. 2162-2176.

134. Haserick, J.R. and D.W. Bortz, *Normal bone marrow inclusion phenomena induced by lupus erythematosus plasma.* J Invest Dermatol, 1949. **13**(2): p. 47-9.

135. Radic, M., et al., *Regulatory and pathogenetic mechanisms of autoantibodies in SLE.* Autoimmunity, 2011. **44**(5): p. 349-56.

136. Weiss, J.E., *Pediatric systemic lupus erythematosus: more than a positive antinuclear antibody.* Pediatr Rev, 2012. **33**(2): p. 62-73; quiz 74.

137. Kumar, Y., A. Bhatia, and R.W. Minz, *Antinuclear antibodies and their detection methods in diagnosis of connective tissue diseases: a journey revisited.* Diagn Pathol, 2009. **4**: p. 1.

138. Saikia, B., A. Rawat, and P. Vignesh, *Autoantibodies and their Judicious Use in Pediatric Rheumatology Practice.* Indian J Pediatr, 2016. **83**(1): p. 53-62.

139. Glerup, M., T. Herlin, and M. Twilt, *Remission rate is not dependent on the presence of antinuclear antibodies in juvenile idiopathic arthritis.* Clin Rheumatol, 2017. **36**(3): p. 671-676.

140. Campanilho-Marques, R., et al., *Prognostic value of antinuclear antibodies in juvenile idiopathic arthritis and anterior uveitis. Results from a systematic literature review.* Acta Reumatol Port, 2014. **39**(2): p. 116-22.

141. Stoll, M.L. and M. Punaro, *Psoriatic juvenile idiopathic arthritis: a tale of two subgroups.* Curr Opin Rheumatol, 2011. **23**(5): p. 437-43.

142. Hugle, B., et al., *Development of positive antinuclear antibodies and rheumatoid factor in systemic juvenile idiopathic arthritis points toward an autoimmune phenotype later in the disease course.* Pediatr Rheumatol Online J, 2014. **12**: p. 28.

143. Iglesias, E., et al., *Non-systemic juvenile idiopathic arthritis outcome after reaching clinical remission with anti-TNF-alpha therapy: a clinical practice observational study of patients who discontinued treatment.* Rheumatol Int, 2014. **34**(8): p. 1053-7.

144. Stoll, M.L., et al., *Elevated IgG autoantibody production in oligoarticular juvenile idiopathic arthritis may predict a refractory course.* Clin Exp Rheumatol, 2011. **29**(4): p. 736-42.

145. Lafyatis, R., et al., *B cell depletion with rituximab in patients with diffuse cutaneous systemic sclerosis.* Arthritis Rheum, 2009. **60**(2): p. 578-83.

146. Pisetsky, D.S. and P.E. Lipsky, *New insights into the role of antinuclear antibodies in systemic lupus erythematosus.* Nat Rev Rheumatol, 2020. **16**(10): p. 565-579.

147. Chepy, A., et al., *Can Antinuclear Antibodies Have a Pathogenic Role in Systemic Sclerosis?* Front Immunol, 2022. **13**: p. 930970.

148. Shao, W.H. and P.L. Cohen, *Disturbances of apoptotic cell clearance in systemic lupus erythematosus.* Arthritis Res Ther, 2011. **13**(1): p. 202.

149. Rosen, A. and L. Casciola-Rosen, *Autoantigens as substrates for apoptotic proteases: implications for the pathogenesis of systemic autoimmune disease.* Cell Death Differ, 1999. **6**(1): p. 6-12.

150. Anderton, S.M., *Post-translational modifications of self antigens: implications for autoimmunity.* Curr Opin Immunol, 2004. **16**(6): p. 753-8.

151. Munoz, L.E., et al., *SLE--a disease of clearance deficiency?* Rheumatology (Oxford), 2005. **44**(9): p. 1101-7.

152. Hall, J.C., L. Casciola-Rosen, and A. Rosen, *Altered structure of autoantigens during apoptosis.* Rheum Dis Clin North Am, 2004. **30**(3): p. 455-71, vii.

153. Rovere, P., et al., *Bystander apoptosis triggers dendritic cell maturation and antigen-presenting function.* J Immunol, 1998. **161**(9): p. 4467-71.

154. Gaipl, U.S., et al., *Clearance deficiency and systemic lupus erythematosus (SLE).* J Autoimmun, 2007. **28**(2-3): p. 114-21.

155. Casciola-Rosen, L.A., G. Anhalt, and A. Rosen, *Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes.* J Exp Med, 1994. **179**(4): p. 1317-30.

156. Rahman, A. and D.A. Isenberg, *Systemic lupus erythematosus.* N Engl J Med, 2008. **358**(9): p. 929-39.

157. Muro, Y., *Antinuclear antibodies.* Autoimmunity, 2005. **38**(1): p. 3-9.

158. Schellekens, G.A., et al., *Citrulline is an Essential Constituent of Antigenic Determinants Recognized by Rheumatoid Arthritis-specific Autoantibodies. 1998.* J Immunol, 2015. **195**(1): p. 8-16.

159. Schellekens, G.A., et al., *The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide.* Arthritis Rheum, 2000. **43**(1): p. 155-63.

160. van Gaalen, F.A., et al., *Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study.* Arthritis Rheum, 2004. **50**(3): p. 709-15.

161. Avouac, J., L. Gossec, and M. Dougados, *Diagnostic and predictive value of anti-cyclic citrullinated protein antibodies in rheumatoid arthritis: a systematic literature review.* Ann Rheum Dis, 2006. **65**(7): p. 845-51.

162. Forslind, K., et al., *Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (anti-CCP).* Ann Rheum Dis, 2004. **63**(9): p. 1090-5.

163. Jansen, L.M., et al., *The predictive value of anti-cyclic citrullinated peptide antibodies in early arthritis.* J Rheumatol, 2003. **30**(8): p. 1691-5.

164. Kastbom, A., et al., *Anti-CCP antibody test predicts the disease course during 3 years in early rheumatoid arthritis (the Swedish TIRA project).* Ann Rheum Dis, 2004. **63**(9): p. 1085-9.

165. Saraux, A., et al., *Value of antibodies to citrulline-containing peptides for diagnosing early rheumatoid arthritis.* J Rheumatol, 2003. **30**(12): p. 2535-9.

166. Vencovsky, J., et al., *Autoantibodies can be prognostic markers of an erosive disease in early rheumatoid arthritis.* Ann Rheum Dis, 2003. **62**(5): p. 427-30.

167. Krishnamurthy, A., et al., *Identification of a novel chemokine-dependent molecular mechanism underlying rheumatoid arthritis-associated autoantibody-mediated bone loss.* Ann Rheum Dis, 2016. **75**(4): p. 721-9.

168. Wigerblad, G., et al., *Autoantibodies to citrullinated proteins induce joint pain independent of inflammation via a chemokine-dependent mechanism.* Ann Rheum Dis, 2016. **75**(4): p. 730-8.

169. Rantapaa-Dahlqvist, S., et al., *Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis.* Arthritis Rheum, 2003. **48**(10): p. 2741-9.

170. Willemze, A., et al., *The ACPA recognition profile and subgrouping of ACPA-positive RA patients.* Ann Rheum Dis, 2012. **71**(2): p. 268-74.

171. Brink, M., et al., *Multiplex analyses of antibodies against citrullinated peptides in individuals prior to development of rheumatoid arthritis.* Arthritis Rheum, 2013. **65**(4): p. 899-910.

172. Kinloch, A., et al., *Synovial fluid is a site of citrullination of autoantigens in inflammatory arthritis.* Arthritis Rheum, 2008. **58**(8): p. 2287-95.

173. Lundberg, K., et al., *Antibodies to citrullinated alpha-enolase peptide 1 are specific for rheumatoid arthritis and cross-react with bacterial enolase.* Arthritis Rheum, 2008. **58**(10): p. 3009-19.

174. Masson-Bessiere, C., et al., *The major synovial targets of the rheumatoid arthritis-specific antifilaggrin autoantibodies are deiminated forms of the alpha- and beta-chains of fibrin.* J Immunol, 2001. **166**(6): p. 4177-84.

175. Syed, R.H., B.E. Gilliam, and T.L. Moore, *Rheumatoid factors and anticyclic citrullinated peptide antibodies in pediatric rheumatology.* Curr Rheumatol Rep, 2008. **10**(2): p. 156-63.

176. Avcin, T., et al., *Prevalence and clinical significance of anti-cyclic citrullinated peptide antibodies in juvenile idiopathic arthritis.* Ann Rheum Dis, 2002. **61**(7): p. 608-11.

177. Brunner, J.K. and F.C. Sitzmann, *Anticyclic citrullinated peptide antibodies in juvenile idiopathic arthritis.* Mod Rheumatol, 2006. **16**(6): p. 372-5.

178. Gupta, R., et al., *Anti-cyclic citrullinated peptide antibodies in juvenile idiopathic arthritis.* Indian J Pediatr, 2010. **77**(1): p. 41-4.

179. Kwok, J.S., et al., *Anti-cyclic citrullinated peptide: diagnostic and prognostic values in juvenile idiopathic arthritis and rheumatoid arthritis in a Chinese population.* Scand J Rheumatol, 2005. **34**(5): p. 359-66.

180. Low, J.M., et al., *Determination of anti-cyclic citrullinated peptide antibodies in the sera of patients with juvenile idiopathic arthritis.* J Rheumatol, 2004. **31**(9): p. 1829-33.

181. Nishimura, K., et al., *Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis.* Ann Intern Med, 2007. **146**(11): p. 797-808.

182. Sun, J., et al., *Diagnostic accuracy of combined tests of anti cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis: a meta-analysis.* Clin Exp Rheumatol, 2014. **32**(1): p. 11-21.

183. Wang, Y., et al., *Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody for juvenile idiopathic arthritis.* J Immunol Res, 2015. **2015**: p. 915276.

184. Lipinska, J., et al., *Anticitrullinated protein antibodies and radiological progression in juvenile idiopathic arthritis.* J Rheumatol, 2012. **39**(5): p. 1078-87.

185. van Rossum, M., et al., *Anti-cyclic citrullinated peptide (anti-CCP) antibodies in children with juvenile idiopathic arthritis.* J Rheumatol, 2003. **30**(4): p. 825-8.

186. Gilliam, B.E., et al., *Measurement of biomarkers in juvenile idiopathic arthritis patients and their significant association with disease severity: a comparative study.* Clin Exp Rheumatol, 2008. **26**(3): p. 492-7.

187. Omar, A., et al., *Anti-cyclic citrullinated peptide (anti-CCP) antibody in juvenile idiopathic arthritis (JIA): correlations with disease activity and severity of joint damage (a multicenter trial).* Joint Bone Spine, 2013. **80**(1): p. 38-43.

188. Berntson, L., et al., *Anti-type II collagen antibodies, anti-CCP, IgA RF and IgM RF are associated with joint damage, assessed eight years after onset of juvenile idiopathic arthritis (JIA).* Pediatr Rheumatol Online J, 2014. **12**: p. 22.

189. Brunner, J. and F.C. Sitzmann, *The diagnostic value of anti-cyclic citrullinated peptide (CCP) antibodies in children with Juvenile Idiopathic Arthritis.* Clin Exp Rheumatol, 2006. **24**(4): p. 449-51.

190. Skare, T.S., et al., *Anti-cyclic citrullinated peptide antibodies in adult patients with juvenile idiopathic arthritis.* J Clin Rheumatol, 2011. **17**(8): p. 421-3.

191. Peckham, H., et al., *Antibodies to Cyclic Citrullinated Peptides in Patients With Juvenile Idiopathic Arthritis and Patients With Rheumatoid Arthritis: Shared Expression of the Inherently Autoreactive 9G4 Idiotype.* Arthritis Rheumatol, 2017. **69**(7): p. 1387-1395.

192. Ringold, S., et al., *Childhood Arthritis and Rheumatology Research Alliance consensus treatment plans for new-onset polyarticular juvenile idiopathic arthritis.* Arthritis Care Res (Hoboken), 2014. **66**(7): p. 1063-72.

193. Gilliam, B.E., A.K. Chauhan, and T.L. Moore, *Evaluation of anti-citrullinated type II collagen and anti-citrullinated vimentin antibodies in patients with juvenile idiopathic arthritis.* Pediatr Rheumatol Online J, 2013. **11**(1): p. 31.

194. Gilliam, B.E., et al., *Evidence of fibrinogen as a target of citrullination in IgM rheumatoid factor-positive polyarticular juvenile idiopathic arthritis.* Pediatr Rheumatol Online J, 2011. **9**: p. 8.

195. Kuna, A.T., et al., *Antibodies to mutated citrullinated vimentin and antibodies to cyclic citrullinated peptides in juvenile idiopathic arthritis.* Clin Chem Lab Med, 2009. **47**(12): p. 1525-30.

196. Lipinska, J., et al., *Anti-MCV and anti-CCP antibodies-diagnostic and prognostic value in children with juvenile idiopathic arthritis (JIA).* Clin Rheumatol, 2016. **35**(11): p. 2699-2706.

197. Morbach, H., et al., *Prevalence of antibodies against mutated citrullinated vimentin and cyclic citrullinated peptide in children with juvenile idiopathic arthritis.* Clin Exp Rheumatol, 2010. **28**(5): p. 800.

198. Tebo, A.E., et al., *Profiling anti-cyclic citrullinated peptide antibodies in patients with juvenile idiopathic arthritis.* Pediatr Rheumatol Online J, 2012. **10**(1): p. 29.

199. Witalison, E.E., P.R. Thompson, and L.J. Hofseth, *Protein Arginine Deiminases and Associated Citrullination: Physiological Functions and Diseases Associated with Dysregulation.* Curr Drug Targets, 2015. **16**(7): p. 700-10.

200. Spengler, J., et al., *Release of Active Peptidyl Arginine Deiminases by Neutrophils Can Explain Production of Extracellular Citrullinated Autoantigens in Rheumatoid Arthritis Synovial Fluid.* Arthritis Rheumatol, 2015. **67**(12): p. 3135-45.

201. Willis, V.C., et al., *Protein arginine deiminase 4 inhibition is sufficient for the amelioration of collagen-induced arthritis.* Clin Exp Immunol, 2017. **188**(2): p. 263-274.

202. Alivernini, S., et al., *Citrullination: the loss of tolerance and development of autoimmunity in rheumatoid arthritis.* Reumatismo, 2008. **60**(2): p. 85-94.

203. Klareskog, L. and A.I. Catrina, *Autoimmunity: lungs and citrullination.* Nat Rev Rheumatol, 2015. **11**(5): p. 261-2.

204. Rubin, B. and G. Sonderstrup, *Citrullination of self-proteins and autoimmunity.* Scand J Immunol, 2004. **60**(1-2): p. 112-20.

205. Klareskog, L., et al., *Immunity to citrullinated proteins in rheumatoid arthritis.* Annu Rev Immunol, 2008. **26**: p. 651-75.

206. Clavel, C., et al., *Induction of macrophage secretion of tumor necrosis factor alpha through Fcgamma receptor IIa engagement by rheumatoid arthritis-specific autoantibodies to citrullinated proteins complexed with fibrinogen.* Arthritis Rheum, 2008. **58**(3): p. 678-88.

207. Harre, U., et al., *Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin.* J Clin Invest, 2012. **122**(5): p. 1791-802.

208. Khandpur, R., et al., *NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis.* Sci Transl Med, 2013. **5**(178): p. 178ra40.

209. Malmstrom, V., A.I. Catrina, and L. Klareskog, *The immunopathogenesis of seropositive rheumatoid arthritis: from triggering to targeting.* Nat Rev Immunol, 2017. **17**(1): p. 60-75.

210. Sokolove, J., et al., *Immune complexes containing citrullinated fibrinogen costimulate macrophages via Toll-like receptor 4 and Fcgamma receptor.* Arthritis Rheum, 2011. **63**(1): p. 53-62.

211. Trouw, L.A., et al., *Anti-cyclic citrullinated peptide antibodies from rheumatoid arthritis patients activate complement via both the classical and alternative pathways.* Arthritis Rheum, 2009. **60**(7): p. 1923-31.

212. Rose, H.M., C. Ragan, and et al., *Differential agglutination of normal and sensitized sheep erythrocytes by sera of patients with rheumatoid arthritis.* Proc Soc Exp Biol Med, 1948. **68**(1): p. 1-6.

213. Ingegnoli, F., R. Castelli, and R. Gualtierotti, *Rheumatoid factors: clinical applications.* Dis Markers, 2013. **35**(6): p. 727-34.

214. Gioud-Paquet, M., et al., *IgM rheumatoid factor (RF), IgA RF, IgE RF, and IgG RF detected by ELISA in rheumatoid arthritis.* Ann Rheum Dis, 1987. **46**(1): p. 65-71.

215. Aletaha, D., et al., *2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative.* Arthritis Rheum, 2010. **62**(9): p. 2569-81.

216. Falkenburg, W.J., et al., *IgG Subclass Specificity Discriminates Restricted IgM Rheumatoid Factor Responses From More Mature Anti-Citrullinated Protein Antibody-Associated or Isotype-Switched IgA Responses.* Arthritis Rheumatol, 2015. **67**(12): p. 3124-34.

217. Williams, R.C., Jr. and H.G. Kunkel, *Rheumatoid factor, complement, and conglutinin aberrations in patients with subacute bacterial endocarditis.* J Clin Invest, 1962. **41**(3): p. 666-75.

218. Toumbis, A., et al., *Clinical and serologic observations in patients with juvenile rheumatoid arthritis and their relatives.* J Pediatr, 1963. **62**: p. 463-73.

219. Eichenfield, A.H., et al., *Utility of rheumatoid factor in the diagnosis of juvenile rheumatoid arthritis.* Pediatrics, 1986. **78**(3): p. 480-4.

220. Flato, B., 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): p. 386-93.

221. Carson, D.A., P.P. Chen, and T.J. Kipps, *New roles for rheumatoid factor.* J Clin Invest, 1991. **87**(2): p. 379-83.

222. Borretzen, M., et al., *Differences in mutational patterns between rheumatoid factors in health and disease are related to variable heavy chain family and germ-line gene usage.* Eur J Immunol, 1997. **27**(3): p. 735-41.

223. Pai, S., L. Pai, and R. Birkenfeldt, *Correlation of serum IgA rheumatoid factor levels with disease severity in rheumatoid arthritis.* Scand J Rheumatol, 1998. **27**(4): p. 252-6.

224. Leadbetter, E.A., et al., *Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors.* Nature, 2002. **416**(6881): p. 603-7.

225. Mannik, M., *Rheumatoid factors in the pathogenesis of rheumatoid arthritis.* J Rheumatol Suppl, 1992. **32**: p. 46-9.

226. Saevarsdottir, S., et al., *Multiomics analysis of rheumatoid arthritis yields sequence variants that have large effects on risk of the seropositive subset.* Ann Rheum Dis, 2022. **81**(8): p. 1085-1095.

227. Diaz-Gallo, L.M., et al., *Four Systemic Lupus Erythematosus Subgroups, Defined by Autoantibodies Status, Differ Regarding HLA-DRB1 Genotype Associations and Immunological and Clinical Manifestations.* ACR Open Rheumatol, 2022. **4**(1): p. 27-39.

228. Leclair, V., et al., *Distinct HLA associations with autoantibody-defined subgroups in idiopathic inflammatory myopathies.* EBioMedicine, 2023. **96**: p. 104804.

229. Zhao, Y. and C. Wallace, *Judicious use of biologicals in juvenile idiopathic arthritis.* Curr Rheumatol Rep, 2014. **16**(11): p. 454.

230. Struglics, A., et al., *Juvenile idiopathic arthritis patients have a distinct cartilage and bone biomarker profile that differs from healthy and knee-injured children.* Clin Exp Rheumatol, 2020. **38**(2): p. 355-365.

231. Sophia Fox, A.J., A. Bedi, and S.A. Rodeo, *The basic science of articular cartilage: structure, composition, and function.* Sports Health, 2009. **1**(6): p. 461-8.

232. Oliviero, F. and R. Ramonda, *Cartilage-derived biomarkers in osteoarthritis.* Indian J Med Res, 2021. **153**(4): p. 413-415.

233. Eschweiler, J., et al., *The Biomechanics of Cartilage-An Overview.* Life (Basel), 2021. **11**(4).

234. Kumavat, R., et al., *Biomarkers of Joint Damage in Osteoarthritis: Current Status and Future Directions.* Mediators Inflamm, 2021. **2021**: p. 5574582.

235. Henrotin, Y., et al., *Osteoarthritis biomarkers derived from cartilage extracellular matrix: Current status and future perspectives.* Ann Phys Rehabil Med, 2016. **59**(3): p. 145-148.

236. Bakker, M.F., et al., *The relation between cartilage biomarkers (C2C, C1,2C, CS846, and CPII) and the long-term outcome of rheumatoid arthritis patients within the CAMERA trial.* Arthritis Res Ther, 2011. **13**(3): p. R70.

237. Dejica, V.M., et al., *Cleavage of type II collagen by cathepsin K in human osteoarthritic cartilage.* Am J Pathol, 2008. **173**(1): p. 161-9.

238. Mullan, R.H., et al., *Early changes in serum type II collagen biomarkers predict radiographic progression at one year in inflammatory arthritis patients after biologic therapy.* Arthritis Rheum, 2007. **56**(9): p. 2919-28.

239. Verstappen, S.M., et al., *Radiographic joint damage in rheumatoid arthritis is associated with differences in cartilage turnover and can be predicted by serum*

biomarkers: an evaluation from 1 to 4 years after diagnosis. Arthritis Res Ther, 2006. **8**(1): p. R31.

240. Cibere, J., et al., *Glucosamine sulfate and cartilage type II collagen degradation in patients with knee osteoarthritis: randomized discontinuation trial results employing biomarkers.* J Rheumatol, 2005. **32**(5): p. 896-902.

241. Pederzoli, S., et al., *Potential value of cartilage and bone soluble biomarkers in evaluating joint damage in juvenile idiopathic arthritis.* Pediatric Rheumatology, 2014. **12**(Suppl 1).

242. Struglics, A., et al., *Aggrecanase cleavage in juvenile idiopathic arthritis patients is minimally detected in the aggrecan interglobular domain but robust at the aggrecan C-terminus.* Arthritis Rheum, 2012. **64**(12): p. 4151-61; author reply 4162-3.

243. Jung, Y.O., et al., *Correlation of sonographic severity with biochemical markers of synovium and cartilage in knee osteoarthritis patients.* Clin Exp Rheumatol, 2006. **24**(3): p. 253-9.

244. Vilim, V., et al., *Serum cartilage oligomeric matrix protein reflects the presence of clinically diagnosed synovitis in patients with knee osteoarthritis.* Osteoarthritis Cartilage, 2001. **9**(7): p. 612-8.

245. Soderlin, M.K., et al., *Antibodies against cyclic citrullinated peptide (CCP) and levels of cartilage oligomeric matrix protein (COMP) in very early arthritis: relation to diagnosis and disease activity.* Scand J Rheumatol, 2004. **33**(3): p. 185-8.

246. Skoumal, M., et al., *Serum levels of cartilage oligomeric matrix protein (COMP): a rapid decrease in patients with active rheumatoid arthritis undergoing intravenous steroid treatment.* Rheumatol Int, 2006. **26**(11): p. 1001-4.

247. Skoumal, M., G. Kolarz, and A. Klingler, *Serum levels of cartilage oligomeric matrix protein. A predicting factor and a valuable parameter for disease management in rheumatoid arthritis.* Scand J Rheumatol, 2003. **32**(3): p. 156-61.

248. Bjornhart, B., et al., *Cartilage oligomeric matrix protein in patients with juvenile idiopathic arthritis: relation to growth and disease activity.* J Rheumatol, 2009. **36**(8): p. 1749-54.

249. Urakami, T., et al., *Clinical significance of decreased serum concentration of cartilage oligomeric matrix protein in systemic juvenile idiopathic arthritis.* J Rheumatol, 2006. **33**(5): p. 996-1000.

250. Iba, K., et al., *Changes in urinary NTX levels in patients with primary osteoporosis undergoing long-term bisphosphonate treatment.* J Orthop Sci, 2008. **13**(5): p. 438-41.

251. Gorska, A., et al., *Bone mineral metabolism in children with juvenile idiopathic arthritis--preliminary report.* Ortop Traumatol Rehabil, 2008. **10**(1): p. 54-62.

252. Hayman, A.R., *Tartrate-resistant acid phosphatase (TRAP) and the osteoclast/immune cell dichotomy.* Autoimmunity, 2008. **41**(3): p. 218-23.

253. Wu, Y.Y., et al., *Serum tartrate-resistant acid phosphatase 5b activity as a prognostic marker of survival in breast cancer with bone metastasis.* BMC Cancer, 2010. **10**: p. 158.

254. *<Tartrate resistant acid phosphatase (TRAP) positive cells in rheumatoid synovium may induce the destruction of articular cartilage.pdf>.*

255. Lau, K.H., et al., *Characterization and assay of tartrate-resistant acid phosphatase activity in serum: potential use to assess bone resorption.* Clin Chem, 1987. **33**(4): p. 458-62.

256. Janckila, A.J. and L.T. Yam, *Biology and clinical significance of tartrate-resistant acid phosphatases: new perspectives on an old enzyme.* Calcif Tissue Int, 2009. **85**(6): p. 465-83.

257. Lamp, E.C. and H.G. Drexler, *Biology of tartrate-resistant acid phosphatase.* Leuk Lymphoma, 2000. **39**(5-6): p. 477-84.

258. Jia, X., et al., *Imputing amino acid polymorphisms in human leukocyte antigens.* PLoS One, 2013. **8**(6): p. e64683.

259. Forre, O., et al., *HLA antigens in juvenile arthritis. Genetic basis for the different subtypes.* Arthritis Rheum, 1983. **26**(1): p. 35-8.

260. Stastny, P. and C.W. Fink, *Different HLA-D associations in adult and juvenile rheumatoid arthritis.* J Clin Invest, 1979. **63**(1): p. 124-30.

261. Fernandez-Vina, M.A., C.W. Fink, and P. Stastny, *HLA antigens in juvenile arthritis. Pauciarticular and polyarticular juvenile arthritis are immunogenetically distinct.* Arthritis Rheum, 1990. **33**(12): p. 1787-94.

262. Thomson, W., et al., *Juvenile idiopathic arthritis classified by the ILAR criteria: HLA associations in UK patients.* Rheumatology (Oxford), 2002. **41**(10): p. 1183-9.

263. Haas, J.P., et al., *Susceptible and protective major histocompatibility complex class II alleles in early-onset pauciarticular juvenile chronic arthritis.* Hum Immunol, 1994. **41**(3): p. 225-33.

264. Ravelli, A., 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): p. 826-32.

265. Ravelli, 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): p. 267-75.

266. Nigrovic, P.A., et al., *Biological classification of childhood arthritis: roadmap to a molecular nomenclature.* Nat Rev Rheumatol, 2021. **17**(5): p. 257-269.

267. Assadi, G., et al., *LACC1 polymorphisms in inflammatory bowel disease and juvenile idiopathic arthritis.* Genes Immun, 2016. **17**(4): p. 261-4.

268. Patel, N., et al., *Study of Mendelian forms of Crohn's disease in Saudi Arabia reveals novel risk loci and alleles.* Gut, 2014. **63**(11): p. 1831-2.

269. Jostins, L., et al., *Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease.* Nature, 2012. **491**(7422): p. 119-24.

270. Franke, A., et al., *Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci.* Nat Genet, 2010. **42**(12): p. 1118-25.

271. Wellcome Trust Case Control, C., *Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls.* Nature, 2007. **447**(7145): p. 661-78.

272. Baldrian, P., *Fungal laccases - occurrence and properties.* FEMS Microbiol Rev, 2006. **30**(2): p. 215-42.

273. Giardina, P., et al., *Laccases: a never-ending story.* Cell Mol Life Sci, 2010. **67**(3): p. 369-85.

274. Barrett, J.C., et al., *Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease.* Nat Genet, 2008. **40**(8): p. 955-62.

275. Torkvist, L., et al., *Analysis of 39 Crohn's disease risk loci in Swedish inflammatory bowel disease patients.* Inflamm Bowel Dis, 2010. **16**(6): p. 907-9.

276. Kaser, A., S. Zeissig, and R.S. Blumberg, *Inflammatory bowel disease.* Annu Rev Immunol, 2010. **28**: p. 573-621.

277. Tejesvi, M.V., et al., *Faecal microbiome in new-onset juvenile idiopathic arthritis.* Eur J Clin Microbiol Infect Dis, 2016. **35**(3): p. 363-70.

278. Hirayama, D., T. Iida, and H. Nakase, *The Phagocytic Function of Macrophage-Enforcing Innate Immunity and Tissue Homeostasis.* Int J Mol Sci, 2017. **19**(1).

279. Sachet, M., Y.Y. Liang, and R. Oehler, *The immune response to secondary necrotic cells.* Apoptosis, 2017. **22**(10): p. 1189-1204.

280. Vernon, P.J. and D. Tang, *Eat-me: autophagy, phagocytosis, and reactive oxygen species signaling.* Antioxid Redox Signal, 2013. **18**(6): p. 677-91.

281. Crawford, E.D. and J.A. Wells, *Caspase substrates and cellular remodeling.* Annu Rev Biochem, 2011. **80**: p. 1055-87.

282. Mahajan, A., M. Herrmann, and L.E. Munoz, *Clearance Deficiency and Cell Death Pathways: A Model for the Pathogenesis of SLE.* Front Immunol, 2016. **7**: p. 35.

283. Webb, T., et al., *Clinical evaluation of a novel chemiluminescent immunoassay for the detection of anti-citrullinated peptide antibodies.* Clin Chim Acta, 2014. **437**: p. 161-7.

284. Pang, S.Y., et al., *Diagnostic performance of anti-citrullinated protein/peptide antibodies in juvenile idiopathic arthritis.* Genet Mol Res, 2016. **15**(2).

285. Syed, R.H., B.E. Gilliam, and T.L. Moore, *Prevalence and significance of isotypes of anti-cyclic citrullinated peptide antibodies in juvenile idiopathic arthritis.* Ann Rheum Dis, 2008. **67**(7): p. 1049-51.

286. Sparchez, M., et al., *Evaluation of anti-cyclic citrullinated peptide antibodies may be beneficial in RF-negative juvenile idiopathic arthritis patients.* Clin Rheumatol, 2016. **35**(3): p. 601-7.

287. Kurreeman, F., et al., *Genetic basis of autoantibody positive and negative rheumatoid arthritis risk in a multi-ethnic cohort derived from electronic health records.* Am J Hum Genet, 2011. **88**(1): p. 57-69.

288. Reed, E., et al., *Presence of autoantibodies in "seronegative" rheumatoid arthritis associates with classical risk factors and high disease activity.* Arthritis Res Ther, 2020. **22**(1): p. 170.

289. Terao, C., et al., *Distinct HLA Associations with Rheumatoid Arthritis Subsets Defined by Serological Subphenotype.* Am J Hum Genet, 2019. **105**(3): p. 616-624.

290. Hunter, D.J., et al., *Biomarkers for osteoarthritis: current position and steps towards further validation.* Best Pract Res Clin Rheumatol, 2014. **28**(1): p. 61-71.

291. Chen, C.J., et al., *Evaluation of the activity of tartrate-resistant acid phosphatase isoform 5b in normal Chinese children--a novel marker for bone growth.* J Pediatr Endocrinol Metab, 2005. **18**(1): p. 55-62.